DIFFERENTIAL EXPRESSION OF ANGIOGENIC FACTORS IN SKIN OF PATIENTS WITH PSORIASIS VULGARIS

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In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

DECLARATION

I hereby declare that the dissertation entitled "**DIFFERENTIAL EXPRESSION OF ANGIOGENIC FACTORS IN SKIN OF PATIENTS WITH PSORIASIS VULGARIS"** is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from April 2013 to July 2014 under the guidance and supervision of **Dr. A. DHANALAKSHMI, M.D**., Associate Professor, Department of Pathology, Coimbatore Medical College.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree (Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

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This is to certify that the dissertation entitled "**DIFFERENTIAL EXPRESSION OF ANGIOGENIC FACTORS IN SKIN OF PATIENTS WITH PSORIASIS VULGARIS**" is a record of bonafide work done by **Dr.S.LAKSHNA** in the Department of Pathology, Coimbatore Medical College, Coimbatore under the guidance and supervision of **Dr. A. DHANALAKSHMI, M.D**., Associate Professor, Department of Pathology, Coimbatore Medical College and submitted in partial fulfilment of the requirements for the award of M.D. Degree (Branch III) in Pathology by The Tamilnadu Dr. MGR Medical University, Chennai.

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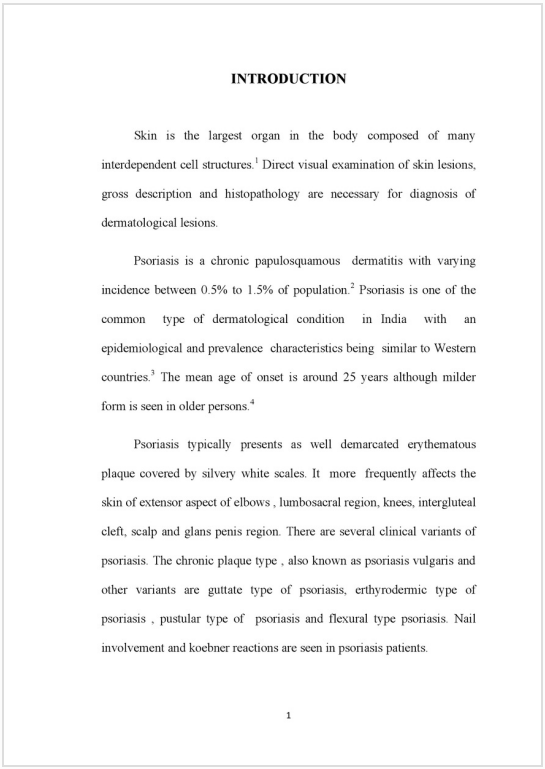

INTRODUCTION

Skin is the largest organ in the body composed of many interdependent cell structures.[1] Direct visual examination of skin lesions, gross description and histopathology are necessary for diagnosis of dermatological lesions.

Psoriasis is a chronic papulosquamous dermatitis with varying incidence between 0.5% to 1.5% of population.[2] Psoriasis is one of the common type of dermatological condition in India with an epidemiological and prevalence characteristics being similar to Western countries.[3] The mean age of onset is around 25 years although milder form is seen in older persons.[4]

Psoriasis typically presents as well demarcated erythematous plaque covered by silvery white scales. It more frequently affects the skin of extensor aspect of elbows , lumbosacral region, knees, intergluteal cleft, scalp and glans penis region. There are several clinical variants of psoriasis. The chronic plaque type , also known as psoriasis vulgaris and other variants are guttate type of psoriasis, erthyrodermic type of psoriasis , pustular type of psoriasis and flexural type psoriasis. Nail involvement and koebner reactions are seen in psoriasis patients.

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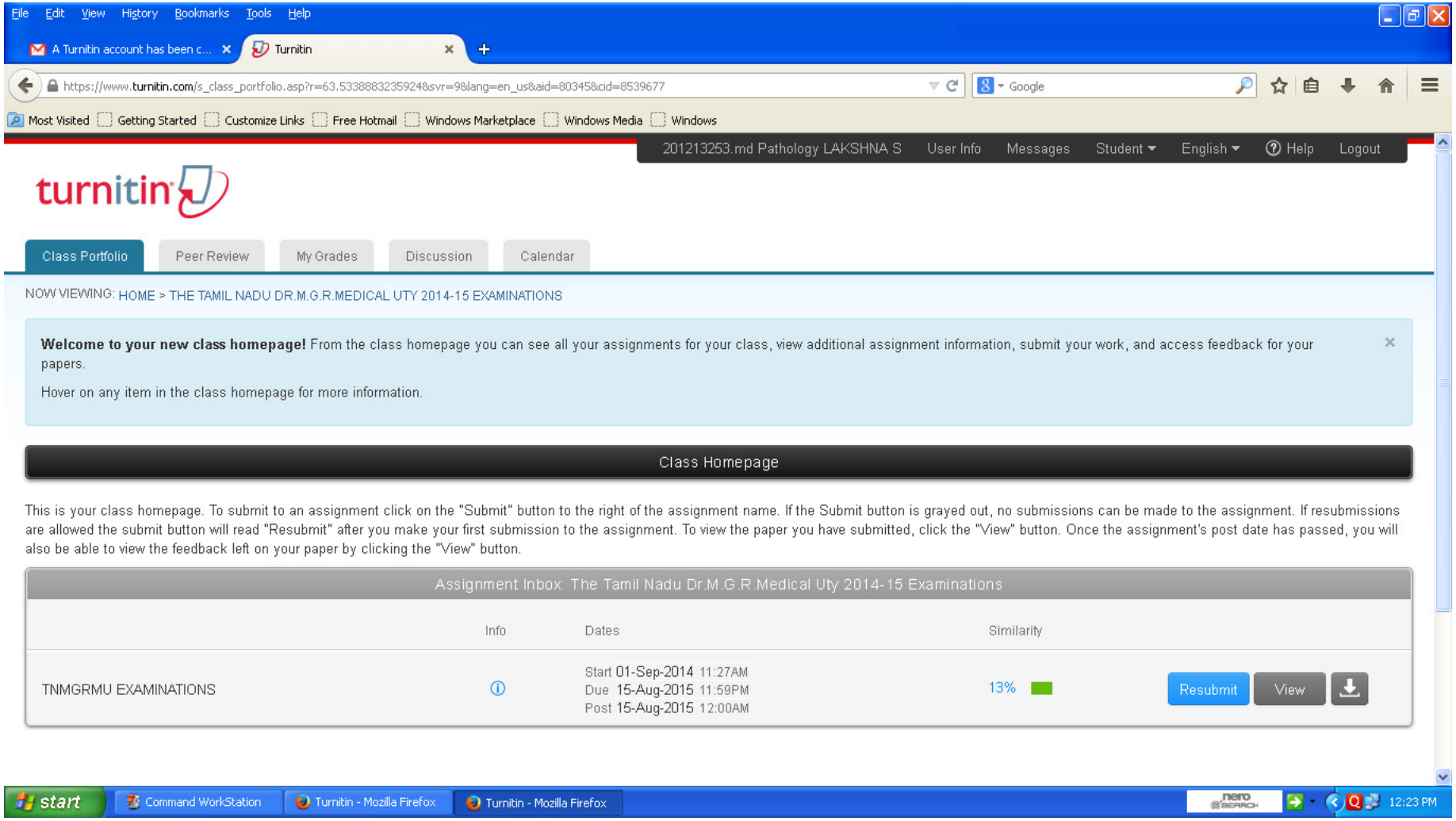
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ABSTRACT

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease characterized by hyper-proliferation, abnormal differentiation, and inflammatory infiltration in epidermis and dermis. Angiogenesis or neovascularization refers to the formation of new capillary vessels from the existing vascular bed .Dermal microvascular expansion with abnormal orientation and dilatation of capillaries in the biopsies of the psoriatic skin revealed that the disease was angiogenesis dependent .

AIM AND OBJECTIVES

- to analyse immunohistochemical expression of angiogenic factors Vascular endothelial growth factor, Von wille brand factor and CD 34 in skin biopsy of patients with psoriasis vulgaris and controls.
- To compare the neovascularisation score of CD 34, VEGF and vWFr in skin biopsy of psoriasis vulgaris cases and controls.
- To correlate the expression of angiogenic factors and psoriasis area and severity clinical index(PASI SCORE) which is a measure of clinical severity .

MATERIALS AND METHODS

This is a case control study carried over a period of 15 months from April 2013 to July 2014.Thirty two psoriasis cases and thirty control healthy skin were studied.Biopsy specimen is taken from skin of newly diagnosed psoriasis and patients who have not taken any treatment two months prior to study. Histopathological examination of psoriasis vulgaris was confirmed. Immunohistochemical expression for VEGF(vascular endothelial growth factor), vonwillebrand factor and CD 34 was studied.

RESULTS

VEGF expression in epidermis was significantly higher in icases when compared to control skin (p=<o.o1).CD 34 expression was significantly upregulated in cases when compared to controls.($p<0.01$). Whereas only weak expression of vonwillebrand factor was observed in both cases and controls. Significant correlation between the expression of VEGF and PASI score($r=0.944;p<0.05$), and expression of CD 34 and PASI score was observed($r=0.942;p<0.05$).

CONCLUSION

Significant overexpression of VEGF and CD 34 was noted in cases when compared to controls.The keratinocytes in the psoriatic skin lesions were recognized as a source of pro-angiogenic cytokines which induce angiogenesis,

namely the vascular endothelial growth factor (VEGF) and other growth factors which promotes micrangiopathic modifications in psoriatic plaque. Angiogenesis plays an important role in genesis and development of psoriasis vulgaris. Therefore development of targeted anti- angiogenic therapy might be beneficial for this chronic disabling dermatological disease.

INTRODUCTION

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The characteristic histopathological features are acanthosis, regular downward elongation of rete ridges, spongiform pustules and munro'smicroabscesses. Papillary dermis shows edema and tortuous dilated capillaries. These dilated tortuous capillaries are the source of bleeding points when the scales are scrapped off from the plaques.[5]

Psoriasis has a complex pathogenesis[6] characterized by altered keratinocyte proliferation and differentiation, immune mediated inflammation , dysregulated angiogenesis and vascular remodelling.[7] Various factors which play a central and pivotal role in the pathomechanism of psoriasis are Th1type of cell, Th17 cell, antigen presenting cell , Langerhan type of cells, natural killer cell , keratinocytes and macrophages and various cytokines of Th1 type.[6]

New blood vessel formation is seen in early stages of psoriatic lesions and neovascularisation disappears with clearance of skin disease.[8] Various angiogenic mediators such as vascular endothelial growth factor, hypoxia inducible factor, and several proangiogenic cytokines like tumour necrosis factor , interleukin 17 and interleukin 8, are increased in lesional skin of psoriasis.[8]

Psoriasis usually has a chronic course, albeit spontaneous or treatment induced remissions do occur.

In present study neovascularisation score in normal skin biopsy as compared to skin biopsy of psoriasis vulgaris patients is done using CD 34. Also the expression of VEGF-vascular endothelial growth factor and vonwillebrand factor in epidermis of normal skin biopsy compared to skin biopsy of psoriatic patients is done. This attempts to prove that vascular changes appears to be significant in pathomechanism of psoriasis. Thus angiogenesis appears to contribute to the pathogenesis of psoriatic lesions.[9]

Anti angiogenic agents may be potential targeted therapy in the future.

The present study encompasses differential immunohistochemical expression of angiogenic factors namely vascular endothelial growth factor (VEGF) , Von willebrand factor and CD 34 in lesional skin of patients with psoriasis vulgaris.

Aims and objectives

AIMS AND OBJECTIVES

- to analyse immunohistochemical expression of angiogenic factors Vascular endothelial growth factor, Von wille brand factor and CD 34 in skin biopsy of patients with psoriasis vulgaris and controls.
- To compare the neovascularisation score of CD 34, VEGF and vWFr in skin biopsy of psoriasis vulgaris cases and controls.
- To correlate the expression of angiogenic factors with psoriasis area severity index(PASI SCORE) which is a measure of clinical severity .

Review of Literature

REVIEW OF LITERATURE

ANATOMY AND HISTOLOGY OF SKIN

Understanding normal skin histology is of prime importance to identify cutaneous pathology. It has two histologically and anatomically separate layers namely the epidermis and the dermis. But epidermis and dermis are functionally interdependent. There is subcutaneous adipose tissue below the dermis.

The skin has many functions including mechanical protection, sun protection, temperature regulation, immune related functions, sensory perception and nutrient metabolism.[10] The epidermal keratinocyte has been recognised as a potent source of immunogenic molecules such as interleukins, colony stimulating factors , interferons, transforming growth factors, tumour necrosis factor and growth factors[11]

EPIDERMIS

Epidermis is predominantly composed of stratified squamous epithelial cells known as keratinocytes. Other types of cells in epidermis are Langerhans cells, Neuroendocrine cells (Merkel cells) , unmyelinated axons and melanocytes. Keratinocytes differ from other cell types by large size, abundant stainable cytoplasm and intercellular bridges.[10]

The keratinocytes are organised into four layers – stratum basale , spinosum, granulosum, lucidum and corneum.

STRATUM BASALE (basal layer)

The basal cell layer is the lowermost layer of epidermis. It has single row of cells of cuboidal type. These cells have round to ovoid nuclei with basophilic cytoplasm and often contain melanin pigmentation from adjacent melanocytes.[10] The basal layer rests on a basement membrane which separates epidermis from dermis. Basal layer is composed of mitotically active cells which give rise to other keratinocytes.

Individual cells are attached to each other by desmosomes and attachment to basement membrane by hemidesmosomes.[10] Cells in the basal layer contain intermediate filaments and they increase in number as they move upward.

STRATUM SPINOSUM (squamous layer)

This second layer which consists of four to six rows of cells is just above the basal layer. Cells in this layer shrink during routine histologic preparations. Thus the intercellular spaces appears to form numerous cytoplasmic extensions or spines. The spines represent the sites where desmosomes are anchored to bundles of tonofilaments. These

tonofilaments provide tensile strength against abrasion of epidermis. The intercellular spaces contain acid mucopolysaccharides and neutral mucopolysaccharides. Hyaluronic acid an important component of acid mucopolysaccarides, is abundant in intercellular spaces of stratum spinosum.[12]

STRATUM GRANULOSUM (granular layer)

This third layer consists of three to five rows of flattened cells. This layer is seen just above stratum spinosum. Cells in this stratum granulosum are filled with dense keratohyaline granules. The granular cell layer is the mature keratin forming transition zone of epidermis.[10] Dissolution of nucleus and cell organelles is starts from this granular cell layer. Diffusely staining lysosomal enzymes in this layer plays an important role in autolytic changes in this layer.[13]

STRATUM LUCIDUM

Stratum lucidum consists of row of tightly packed flattened cells that lack nuclei or organelles. This layer is seen only in thick skin. Cells in this layer contains densely packed keratin filaments.

STRATUM CORNEUM (horny layer)

The horny layer is the superficial layer of the epidermis and consists of flattened dead cells. These cells are anucleate and filled with

soft keratin. The superficial keratinised cells are shed or desquamated continuously and replenished by cells arising from deeper most basal layer or stratum basale. The horny cytoplasm of these cells contain cystine disulfide bonds, these shrink on formalin fixation and form a shell along cell membrane resulting in basket weave appearance in routine histologic sections.[14]

DERMIS

Dermis is seen just below epidermis and consists of connective tissue fibers and cellular components of epidermis. The cellular components are fibroblasts,dermal dendritic cells ,macrophages and mast cells. The extracellular components are collagen,elastic fibers and ground substance.[10]

The pale staining narrow zone of connective tissue just below the dermis is papillary dermis. The papillary dermis indents the basement membrane of epidermis to form dermal papillae. The reticular layer of dermis comprises the rest of dermis. The reticular dermis forms the bulk of dermis. The reticular dermis predominantly consists of dense connective tissue.

The dermal connective tissue consists of numerous blood vessels , nerves and lymph vessels. The dermis is highly vascular. In dermis there

are numerous sensory receptor which are meissner's corpuscles located closer to dermal papillae and pacinian corpuscles which are found deeper in connective tissue of dermis . Though skin appendages such as hair follicle and sweat glands develop from epidermis ,they are located in dermis.

HISTORICAL PERSPECTIVE OF PSORIASIS

Psoriasis is one of the common dermatological disease . Thus it is justified to have some knowledge in history of psoriasis.

The history of psoriasis, remained speculative for the time before Willan (1757-1812), and reliably assigned only for the last 200 years. Robert Willan (1757- 1812) described different types of psoriasis. Hebra (1806-1880) gave a morphological definition, in which histopathology feature was also taken into account.

In the early 20th century, the biochemistry and histochemistry of psoriasis provided new insights. In the second half of the 20th century, immunocytochemistry and immunology threw some light on the pathogenesis of the disease. At the end of the 20th and at the beginning of the 21st century, advent of genetics and immunology have opened up new therapeutic approaches due to the interesting insights into the pathogenesis of psoriasis.[15]

PSORIASIS

Psoriasis is a one of the common , recurrent chronic inflammatory dermatological disease which affects about 2 % of the caucasian population[16] and results in severe impairment of quality of life.

PREVALENCE

The prevalence of psoriasis differs in various parts of the world ranging from 0% to 11.8%.[17,18,19,20.] The prevalence of psoriasis in India ranges from 0.44% to 2.8%. The disease is two times commonly seen in males when compared with females.

GENETIC BASIS OF PSORIASIS

The molecular genetics of psoriasis is complex and multiple genes are involved. Genetic transmission plays an important role in etiopathogenesis of psoriasis. There are seven major susceptibility loci being reported for psoriasis. Many studies have shown that the major susceptibility locus is in chromosome 6p21, known as PSORS1 and is represented in most of the populations. [21-26]An association with various loci was also reported on chromosomes 1p (PSORS7)[25], 1q (PSORS4)[27], 3q (PSORS5)[28], 4q (PSORS3)[29], 17q (PSORS2)[30], and 19p (PSORS6)[31]. Familial clustering of psoriasis cases are noted .[3] Familial

incidence of psoriasis is higher in childhood psoriasis than adult onset psoriasis.[32,33,34]

Psoriasis vulgaris is associated with certain HLA antigens.[3] Psoriasis is associated with HLA A1, B17 and Cw6.[35] Association with HLABw57 and DR7 is seen in South India.[36] HLA Cw *0602 is the main allele with higher frequency in North India.[37]

AGE DISTRIBUTION IN PSORIASIS

Psoriasis tends to occur more commonly in third or fourth decade.[3] The age of onset for psoriasis is bimodal distribution was recognised in many studies. The age for the first clinical presentation of psoriasis ranges from 15 to 20 years of age,and a second peak of onset which occurs at age of 55 to 60 years.[38-41] Two types of psoriasis are seen, type one and two, that can be distinguished by a bimodal age distribution. Type 1 psoriasis has its onset by the age of 40 years; Type II psoriasis has its onset after 40 years of age. Type I disease accounts for more than 75% of cases.[41] Patients with earlier age of onset that is the type I psoriasis have a severe disease and many relatives are affected compared to patients with later onset disease or the type II psoriasis. In psoriasis cases with early onset of skin lesions, strong associations are being reported with human leucocyte antigen Cw6

TRIGGER FACTORS

Various modifiable risk factors predispose an individual to develop psoriasis or exacerbate the already existing psoriatic disease. The modifiable risk factors includes smoking, intake of alcohol, obesity, dietary habits, several types of infection ,drug intake and stress.[42-51] The actual mechanism which aggravates psoriatic skin lesions is yet to be elucidated;

Acute bacterial and viral infections are associated with onset of psoriasis or flaring up of the disease. Streptococcal infection is also an aggravating factor for guttate psoriasis, more commonly in childhood age group and younger adults. Human immunodeficiency virus is also associated with an onset of severe plaque type of psoriasis and does not respond to standard therapy or medications.

Various drugs which includes beta blockers, lithium, antimalarial drugs, tetracycline, nonsteroidal antiinflammatory drugs and withdrawal of steroids are also associated with the onset of psoriasis or flaring up of the disease. Drug induced psoriasis is likely to occur in patients with no past history of psoriatic skin lesions which regresses and clears after withdrawal of the causative medication. Drug aggravated psoriasis occurs in patients who already have a history of psoriatic skin lesions and progresses even after withdrawal of the causative drugs. The actual

mechanism of action by which a drug aggravates psoriasis is not clearly understood. Studies suggest that the mechanism by which beta blocker induced psoriatic skin lesion occurs is due to blocking of epidermal beta 2 receptor that leads to reduced cyclic adenosine monophosphate in the epidermis and hyperproliferation of keratinocytes .

The current thought in lithium aggravating psoriasis is by inhibition of inositol monophosphate, resulting in decrease of intra cellular calcium levels and enhanced proliferation of epidermal keratinocytes. Also the lithium increases tumor necrosis factor alpha production and IFN - gamma production in the epidermal cells and they play a major role in psoriasis.

Antimalarial drugs exacerbate already existing psoriasis in 40% of patients. It acts by inhibition of the enzyme transglutaminase and causes proliferation of keratinocytes. The relevance of antibiotic induced psoriatic skin lesions is still a controversy. The tetracycline class of antibiotics exacerbates psoriasis through decrease in intracellular cyclic adenosine monophosphate. Finally, NSAIDs inhibit the arachidonic acid metabolism that leads to accumulation of leukotrienes and these leukotrienes aggravate psoriasis.

Knowledge regarding the drugs that induces or aggravates psoriasis is of prime importance to prevent the precipitation of the

disease. Psychological stress disorder is also a trigger factor for psoriasis[42,52-55].

PATHOGENESIS OF PSORIASIS

Cells like T lymphocyte cell, macrophages, (APC) antigen-presenting cells, natural killer cells, epidermal keratinocytes, Langerhans' cell, , various types of Th1 cytokines and various growth factors such as (VEGF), vascular endothelial growth factor, (KGF) keratinocyte growth factor play a major role in pathomechanism of psoriasis[6]. There is need to know current concepts and pathomechanisms of psoriasis

CELLULAR BASIS - PSORIASIS

The psoriatic disease commences with the activation of T lymphocyte cell towards a gene product or an unknown antigen. Activation of T lymphocyte depends upon binding of Tcell with antigen presenting cell (APC) followed by expression of the T cell receptor also known as TCR.TCR identifies the peptide presented by the antigen presenting cell within the groove of Major histo compatibility complex. This antigen stimulated activation of T cell results in conversion of naive T lymphocyte into an antigen specific T lymphocyte, that develops into a memory type of cell which circulates in the whole body . Followed by the activation of these T cells, an array of cytokines that is granulocyte macrophage colony stimulating factor (GMCSF),

EGF,interleukin-8, interleukin-12, interleukin-23, interleukin-17, interleukin-1, interleukin-6, Fractalkine, tumor necrosis factor-α and others are produced by these activated T lymphoctes. Due to the effect of the above cytokines there is proliferation of epidermis and hyperplasia of epidermis, migration of neutrophils , increase in response of Th-1 cell type, upregulation of adhesion molecules and angiogenesis,[6] .

ROLE OF KERATINOCYTE

The type of cell which is responsible for the onset of psoriasis is still a controversy. Various investigations concentrated on epidermal keratinocytes. Aberrant activation of keratinocytes and metabolism of keratinocytes in the epidermis, leads to increased proliferation of keratinocytes and they are the characteristic features in lesional psoriatic skin[56]. Psoriatic skin has eight times shortened turnover for keratinocytes due to enhanced keratinocyte proliferation [57.]

Studies have shown that in transgenic mice, deletion of the activator protein 1 family members such as Jun B and C Jun especially in keratinocytes of basal layer causes an inflammatory skin disease simulating psoriatic skin lesions supporting the prime role of keratinocytes triggering psoriatic skin lesions[58]. Recent studies show that in experimental mice ,the psoriatic skin lesions are associated with altered expression of transcription factors of the activator protein-1 in

the epidermal cells. The cell cycle time for hyperproliferating keratinocytes in psoriasis is of shorter duration. The maturation and shedding of epidermal keratinocyte takes atleast 26 days in normal skin, whereas it takes only 4 days for the epidermial keratinocyte of psoriatic skin lesions[59]. Growth factors produced by various types of cell, are known to control the marked keratinocyte proliferation.

KERATINOCYTE AND IMMUNE SYSTEM CROSS TALK

There is a complex interaction between immune system and keratinocytes as basic steps in the pathogenesis of psoriasis[60-65]. Studies in transgenic mice shows that ubiquitous activation of nuclear factor kappa beta (NFkb) transcription factor, which potentially induces inflammatory responses, which results in development of skin lesions resembling psoriasis, which includes hyperkeratosis, acanthosis, parakeratosis as well as dilatation of dermal capillaries[64]. This psoriatic skin lesion is dependent on the simultaneous nuclear Factor kappa beta activation in keratinocytes and T cells, because selective activation of the transcription factor in keratinocytes or in T cells only is not adequate enough to produce these specific dermatological and histopathological changes. This shows the prime importance of interaction between keratinocytes and T cells in pathogenesis of psoriasis. The Langerhans

cells and Tcells infiltrates into the epidermis, and they come in contact directly with epidermal keratinocytes.

The mononuclear cells which infiltrates the dermis secretes chemical mediators that induces the proliferation of keratinocytes and endothelium. The dermal tissue of psoriasis skin lesion is infiltrated by CD4 + T helper type of cells and these T helper cells secretes proinflammatory cytokines such as interferon-IFN-γ,interleukin-17 and tumor necrosis factor alpha. [66,67] . Also the increased levels of cytokines such as IL-8, IL-6 and keratinocyte growth factor [transforming growth factor-alpha] are seen in psoriasis skin lesions[60,65,68]. There is an intense cross-talk between cells of immune system and keratinocytes that establishes an interactive cytokine heirachy,which is responsible for the development of psoriasis.

ROLE OF T CELL AND T CELL ACTIVATION

In the early skin lesions of psoriasis , the dermis is predominantly infiltrated by CD4 + T helper type of cells, that produces interferon gamma and Interleukin -17, but not Interleukin - 4 or IL-10[66,69-71]. Therapy with systemic drugs with cyclosporine A results in impairment of cytokine production and T lymphocytes activation , and this improves psoriasis [72]. The importance of role of T cells which promotes psoriasis is substantiated by clinical observations from psoriatic patients with

haematological malignancy, who are cleared of disease or obtained remission after bone marrow transplantation from healthy donor without any history of psoriasis[73]. Some of the patients developed psoriatic skin lesions initially after the bone marrow transplantation from donors suffering from psoriatic skin lesions[74].

Therapy for psoriatic lesions with monoclonal antibody targeted against CD 4 molecules improves skin lesion, whereas therapy with monoclonal antibody against the CD 8 molecules, does not improve psoriasis[75-77]. Also skin xenograft models on Severe Combined Immuno Deficiency mice shows that populations which includes autologous Interferon -γ- producing CD 4 T helper cells can produce psoriatic skin lesions in healthy grafts from patients with psoriatic skin lesion but autologous transfer of CD8 T helper cells from same patients with history psoriasis could not induce the disease[78]. IFN-γ-producing Th1 cells and IL-17-producing Th 17 cell plays pivotal role for causing psoriasis[67,71,79,80] . The expression of both the Th17 cell promoting cytokine Interleukin 23 and the cytokine IL-22 which is associated with Th17 cell in psoriatic skin, supports that both Th1cell and Th 17 cell are responsible for the manifestation of psoriatic skin lesions[71,81,82].

Thus psoriasis is a Th 1cell /Th17 cell mediated autoimmune inflammatory condition. For the sustained inflammation in

psoriatic skin lesion, the disease inducing Th1 cells and/or Th17 cells either proliferates in situ or migrates to dermis – the target organ from the peripheral site . The process also depends upon close interaction between the inflammatory Thelper 1/Thelper 17 cells within the dermal microvascular bed. There is an interaction between the lymphocyte function associated antigen - LFA-1 on the lymphocytes and the intercellular adhesion molecule - ICAM-1 on the endothelial cell which mediates adhesion of leukocytes to the endothelial cell which is supposed to be prerequisite for extravasation of leucocytes. In inflammatory diseases, ICAM-1 is markedly expressed on the endothelial cells of vascular structures.

One school of thought is that there is abnormal regulation of T lymphocyte and also interaction between the epidermal cells and an array of cytokines are involved in pathogenic basis of psoriasis.[83,84.] When the primary defect is in epidermal keratinocytes,injury in any form either chemical or physical damage to the defective keratinocytes induces the production of cytokines followed by release of cytokines which results in antigen independent activation of the T lymphocyte . This activation leads to release of various other cytokines which is followed by epidermal keratinocyte proliferation, T lymphocyte infiltration and then inflammation.

Studies by Chang *et al* [85] demonstrates that the cytokines secreted by keratinocytes of psoriasis increases activation of T lymphocyte to a significant level than the cytokines secreted from keratinocytes of normal skin. One school of thought is that only keratinocytes of psoriasis skin lesions respond to information from activated T lymphocyte with hyperproliferation, because of their specific type of receptors or specific signal transduction mechanisms[84].

Several studies shows that there is alteration in basement membrane structures and a heirachy of cytokines especially Thelper 1 type were involved in different stages of pathogenesis of psoriasis.[83,86,87.]

ANGIOGENESIS

Angiogenesis in psoriasis is a cofactor as well as induces development of psoriasis . Several changes in the superficial microvessels in psoriatic skin lesions results in an angiogenic phenotype. Proangiogenic cytokines like tumor necrosis factor, VEGF , hypoxia inducible factor , Interleukin 8 and angiopoietins, are increased in lesional skin of psoriasis [88,89]. A pro-angiogenic role is related to the T helper 17 cell- cytokine interleukin 17[90,91]. Angiogenesis is balanced by interaction between proangiogenic stimuli and antiangiogenic stimuli and the expression of anti angiogenic factor expression is modulated

during development of psoriasis. The epidermal keratinocytes isolated from psoriasis skin lesions shows decreased expression of thrombospondin-1 (TSP-1), an endogenous inhibitor of angiogenesis. Thrombospondin inhibits endothelial Cell migration and proliferation, new vessel formation and proliferation of tumour cells[92,93,94]. In normal healthy skin, production of thrombospondin -1 by basal epidermal cells maintains the separation between the avascular epidermis and vascular dermis[95,96].

These findings put together suggests that the involvement of angiogenesis in pathogenesis of psoriasis. Physiological angiogenesis is seen transiently during healing of wounds, pregnancy or the menstrual cycle. Pathological angiogenesis is seen in conditions like neoplastic growth of tumour and chronic inflammatory conditions, which are seen in diseases like rheumatoid arthritis or psoriasis[89,97-100].

Keratinocyte is one of the maximum source of proangiogenic cytokines such as vascular endothelial growth factor, interleukin 8 ,but actual mechanism of angiogenesis in psoriasis is not yet known. In the lesional skin of psoriasis vulgaris , endothelial cells become swollen and gets activated which shows enlarged Golgi bodies and Weibel Palade bodies.[101]Activated endothelial cell migrates, sprouts , and lays a basement membrane with pericytes which is a structural support to form a

neo vascular structure.[102] Activated and swollen endothelial cells leads to widened inter-cellular spaces and dilatation of dermal blood capillaries.

The lesional skin capillaries adopts a venous type, which includes bridging of fenestrations, and expression of E selectin, this helps for easier migration of leucocytes into the epidermis and dermis.[88]

DEFINITION OF ANGIOGENESIS

Angiogenesis means new vessel formation from the preexisting blood capillaries. It is seen during embryogenesis whereas it is absent in many of the adult tissues. Angiogenesis takes place in two different manners : (a) the sprouting type – in which newer blood vessels sprouts from preexisting blood capillaries and (b) the non sprouting type angiogenesis or also known as intussusception,in which there is division of preexisting capillaries by trans-capillary pillars[103,104].

SPROUTING OF NEW VESSELS

Sprouting type of angiogenesis is initiated by the activation of vascular endothelial cells through various factors like vascular endothelial growth factor or basic fibroblast growth factor . The following are the steps of angiogenesis

- Dilatation of blood vessels
- increased vascular permeability
- destabilization of blood capillaries already existing,
- degradation of the extracellular matrix
- Endothelial Cell migration and proliferation
- Formation of vascular lumen and maturation of vessels by recruitment of peri-vascular supporting cells[105,106].

Increased vascular permeability results in leaking of plasma proteins that provides a temporary matrix for migration of endothelial Cells , also it requires the destruction of the extracellular matrix by protease enzymes like matrix metallo-proteinases and plasminogen activators. This also requires temporary destabilization of blood capillaries by dissolution of inter-endothelial and peri-endothelial cell contacts. Extra Cellular Matrix degradation results in release of proangiogenic factors vascular endothelial growth factor, insulin-like growth factor and basic fibroblast growth factor and are stored in the extracellular matrix and hence promotes angiogenesis.

The migration of endothelial cell is guided by the amount of angiogenic cytokines, which includes the expression of integrins, cellular adhesion molecules on the endothelial cell surface, and interacts

with extracellular matrix components. This newly formed immature vascular structures acquires a vascular lumen and matures by the recruiting the supporting cells like pericytes or the smooth muscle cell. In a mature and stable blood vessel, the endothelial cells can survive for many years.

MICROVESSEL CHANGES IN PAPILLARY DERMIS IN PSORIASIS

Psoriasis skin lesions begins with neoangiogenesis in superficial dermis. Dermal papillary capillaries shows prominent dilatation, increased tortuosity, permeability, and also shows elongation which is prominent.[107-109].These morphological changes occur before epidermal hyperplasia becomes evident[107,110].The microvascular changes in early stages of psoriatic lesions correlates with increased cutaneous vascular flow also in the neighbouring perilesional areas[111]. Electron Microscopy reveals ultrastructural changes in the capillaries in the dermis.

In normal skin, capillaries show an arterial type, whereas psoriatic plaques exhibit characteristic features of venous type like single or multilayered basement membrane with bridging of fenestrations in the endothelium[112]. Venous capillary loops returns to arterial capillary loop after therapy[112]. Normalization of the superficial dermal vasculature and capillary loops is followed by normalization of

epidermis[109]. Apart from the morphological changes, the dermal microvasculature in psoriatic skin shows an enhanced expression of inflammatory associated adhesion molecules such as E-selectin, intercellular adhesion molecule 1 and vascular cell adhesion molecule 1.

The adhesion molecules allows firm binding of leukocytes to the endothelial cell [113], which is of prime importance for lymphocyte extravasation and inflammatory response. Migration and proliferation of endothelial cells seem to be important characteristic features of angiogenic endothelial Cells. Endothelial cells show increased proliferation in psoriatic plaques[109,114] proved by autoradiography and immunohistochemistry [115]. For migration endothelial cells utilizes temporary adhesion to constituents of the extracellularmatrix, and is mediated by the integrins which are present on the surface of endothelial cells.

The Integrins are heterodimeric type of transmembrane proteins and it activates intracellular signalling cascades on adhering to the corresponding ligands. Many of the integrins modulates the proangiogenic responses [116]. Among these type of integrins, $\alpha_V\beta3$ is expressed at lower levels on inactive and quiet vessels. The $\alpha_V\beta3$ integrin functions as endothelial cell receptor for von Willebrand factor(vWFr), fibrinogen and fibronectin[117]. In angiogenesis, endothelial cell $\alpha_V\beta3$

expression is markedly increased which may be due to inflammatory conditions or from neoplastic tumour growth.[98,99,118-119]. The supression of angiogenesis *in vivo* by peptides or monoclonal antibody antagonists of $\alpha_V\beta3$ and this indicates its important role in neovascularization[118,119]. In psoriasis, enhanced $\alpha_V\beta3$ expression on endothelial cell is noted. The superficial microvessels of psoriasis skin lesions show increased $\alpha_V\beta3$ levels compared with healthy normal skin[120,121] Angiogenesis could be a reason for the proliferation of the superficial dermal microvessels in psoriatic skin lesions.

PROANGIOGENIC FACTORS IN PSORIASIS

Angiogenesis appears to be one of the prime factors of psoriatic skin lesion , several research works focuses on identifying the proangiogenic cytokines in psoriasis skin lesions. Evidence for keratinocytes producing proangiogenic signals comes from an observation that compared the angiogenic property of conditioned media from epidermal keratinocytes obtained from either psoriasis skin lesion or non lesional skin of psoriasis patients[122]. Conditioned Media from psoriasis skin lesions or non lesional epidermal keratinocyte , activated and enhanced the endothelial cell migration *in vitro* which revealed strong neoangiogenic activity in *in vivo*. Whereas Conditioned Media from epidermal keratinocytes of healthy donor skin revealed no

proangiogenic response. There is a large array of proangiogenic factors, which includes vascular endothelial growth factor, hypoxia inducible factor, angiopoietin, Tumor necrosis factor alpha, Transforming growth factor α, Interleukin 8 and interleukin 17 .

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Vascular endothelial growth factor and their high affinity tyrosine kinase receptor vascular endothelial growth factor receptor -1 and- 2 are primarily involved in vessel embryogenesis and adult new vessel formation. VEGF was initially known as vascular permeability factor [123.] . The active form is a homodimeric type of glycoprotein. VEGFR 1 and VEGFR 2 are expressed by endothelial cell. Vascular endothelial growth factor binding to VEGFR 1 or 2 receptors results in activation of receptors and intracellular signal transduction[124-126]. Vascular endothelial growth factor induced cell proliferation, survival, migration, and increased vascular permeability are essentially transduced by vascular endothelial growth factor receptor 2[127].

VEGF induced angiogenesis contributes to the pathomechanism of psoriatic skin lesions. *In situ* hybridization and immunohistochemical studies reveals that a strong upregulation of vascular endothelial growth factor mRNA and expression of proteins in the cells of epidermis with

increased expression of VEGFR 1 and VEGFR 2 on endothelial cells present in the dermis [128]. As Transforming growth factor α induces secretion and expression of vascular endothelial growth factor by epidermal cells *in vitro*[130] and is overexpressed by suprabasal epidermal cells of psoriasis skin lesions, transforming growth factor α may be a reason for the up-regulation of epidermal VEGF in psoriasis skin lesions.

Serum from patients with psoriatic skin lesions showed increased vascular endothelial growth factor levels. Also serum vascular endothelial growth factor levels correlates with disease severity[129-131]. Also single nucleotide polymorphisms of the vascular endothelial growth factor gene correlates with pathogenesis of psoriasis [132,133] and this suggests that VEGF represents a modified gene in the aetiopathogenesis of psoriatic skin lesions.

The role of vascular endothelial growth factor in pathophysiology of psoriasis was tested using mice overexpressing vascular endothelial growth factor in epidermis[134,135] . Selective overexpression of VEGF in basal keratinocytes lead to a long standing inflammatiory dermatological disease with increase in number of dilated tortuous capillaries, expressing enhanced levels of VEGFR 1 and VEGFR 2, higher number of mast cells in the superficial portion of the dermis and enhanced leukocyte rolling and adhesion[134]. Older K14VEGF animals

spontaneously developed an skin lesion with many features simulating psoriatic skin lesion which includes inflammatory infiltrates of mixed population of CD 4 T lymphocytes, macrophages, mast cells and changes in the microvasculature in the superficial dermis[135]. Transgenic mice treated with the vascular endothelial growth factor antagonist known as VEGF trap remained healthy ,which supports the pivotal role involved by VEGF in causing inflammatory skin disease.

Apart from its central role which causes aberrant angiogenesis in upper portion of the dermis, vascular endothelial growth factor also contributes to keratinocyte proliferation and homeostasis of epidermal barrier[136,137]. Also vascular endothelial growth factor receptor 1 and 2 are detectable in psoriasis skin lesions[136]. As VEGF induces increased expression of VEGFR by keratinocytes *in vitro* and expression of VEGF is upregulated by epidermal keratinocytes, and thus vascular endothelial growth factor contributes to proliferation of keratinocyte in an autocrine fashion.

Psoriasis can also be aggaravated by external injury which is known as Koebner phenomenon and the damage to the epidermal barrier homeostasis stimulates vascular endothelial growth factor expression[137]. Transgenic mice deficient in epidermal expression of vascular endothelial growth factor takes along for time permeability barrier to

recover , decreased density of dermal blood vessel and lacks keratinocyte hyperprloiferation and also angiogenic activity in response to barrier disruption[137]. This elucidates the physiological production of vascular endothelial growth factor plays a role in normal proliferation of epidermal keratinocytes, differentiation and functioning of the normal epidermal keratinocytes.

HYPOXIA INDUCIBLE FACTOR

The cardio-vascular system is generally needed for adequate oxygen supply and nutrient supply. Thus lower oxygen tension is an important trigger factor for angiogenesis. Hypoxia Inducible factor initiates the basic metabolic response to lowered levels of oxygen tension. Hypoxia inducible factor also represents heterodimeric transcription factors consisting of β subunit - aryl hydrocarbon receptor nuclear translocator; and a regulatory al pha subunit (H I F 1α, H I F 2α, H I F 3α)[139-140]. At normal oxygen tension levels, the Hypoxia inducible factor α subunits are synthesized consistently and destructed by the proteasomes . For degradation, the prolyl hydroxylases hydroxylates prolyl residue of the hypoxia inducible factor α subunit , that are active in the normal physiological oxygen concentration level. The hydroxylated form of HIF is identified by the Von Hippel Lindau tumour suppressor protein and leads to ubiquitinylation of hypoxia

inducible factor α and proteasomal destruction. Under hypoxia, prolyl hydroxylases are not active. So consequently, hypoxia inducible factor α subunit is not degraded and the increasing hypoxia inducible factor concentrations leads to translocation in the nucleus. The Hypoxia inducible factor target genes that regulates angiogenesis are VEGF[141-143], VEGFR-1[144] , VEGFR-2 [145], IL-8[146] and Tie-2[147].

In psoriasis skin lesions, HIF 1α and HIF 2α expressions are increased[148]. In epidermal keratinocytes, HIF 1α co locates with VEGF expression, whereas hypoxia inducible factor 2α is expressed in the epidermal keratinocytes and in dermal vasculature. Hypoxia of epidermis and enhanced hypoxia inducible factor expression could result from the robust keratinocyte proliferation and the increased metabolic demands. Also expression of Von Hippel Lindau mRNA and protein are decreased[149].

ANGIOPOIETINS

Apart from the VEGFactor/VEGFactorReceptor signal transduction system, the angiopoietin - Angiopoietin 1 and Angiopoietin 2, and their receptor Tie-2, which is a tyrosine kinase receptor, are all essentially involved in angiogenisis . The Ang – Tie2 system is crucial for the growth, stabilization and maturation of blood vessels[150-152]. Ang-1 induces Tie-2 phosphorylation on adhesion and results in upregulation

of intracellular signal transduction pathways, which leads to stabilization of blood vessels and maintenance during vascular embryogenesis [151]. In adults, the low level Tie 2 activation maintains the resting status of the endothelium[153]. In contrast, the Ang-2 antagonizes the activation of Tie-2 , this causes vessel destabilization [152] and sensitizes preexisting blood capillaries for survival and growth signals. If the proangiogenic signals are absent , Ang 2 results in vessel regression, whereas in the presence of proangiogenic stimuli Angiopoietin 2 results in angiogenic activity.

The Ang–Tie-2 system is upregulated in psoriatic skin lesions[154,155]. Angiopoietin 1 and 2 and Tie-2 are increased in the dermal connective tissue of psoriatic lesional skin[154]. Angiopoietin 1 is also expressed in fibroblasts, mononuclear cells or dendritic Cells, whereas angiopoietin 2 expression appears to be restricted to Endothelial Cell. The predominant decrease of angiopoietin 2 expression following successful treatment suggests a central role of angiopoietin 2 during angiogenesis in psoriasis[154].

The pivotal contribution of signalling of Tie 2 to skin inflammation is demonstrated in a transgenic mouse [155]. Overexpression of Tie 2 results in inflammatory skin condition that reflects certain characteristics features of psoriasis like epidermal keratinocyte hyperproliferation, hyperkeratosis, parakeratosis and inflammatory

infiltration composed of lymphocyte, mast cells, macrophage and enhanced dermal vascularization. Repression of transgenic Tie 2 expression reversed the inflammatory skin lesion entirely.

Apart from the role in angiogenesis, angiopoietin 2 also sensitizes endothelial Cell to these inflammatory signalling cascades like tumor necrosis factor alpha by influencing TNF induced expression of adhesion molecules like intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 on endothelial cells in an autocrine manner, hence facilitates adhesion of leucocytes as well as leucocyte infiltration[156]. Thus, Ang-2 contributes to the inflammatory reaction in the development of psoriatic skin lesion.

CYTOKINES

Many cytokines creates a marked effect on angiogenic activity and influences endothelial cell proliferation , survival and migration or modulates the expression of proangiogenic factors or antiangiogenic mediators. Among the cytokines with proangiogenic activity, tumor necrosis factor , Interleukin-8 and Interleukin 17 are expressed in skin of psoriatic lesions .

TUMOUR NECROSIS FACTOR (TNF)

TNF is the first member of the tumor necrosis factor cytokines family and it is produced as a transmembrane precursor protein. TNF is cleaved proteolytically into a soluble type of protein. It stimulates intracellular signalling by either adhering to p55 tumor necrosis factor receptor with ubiquitous type of expression or p75 tumor necrosis factor receptor 2 with a constrained type of expression by cells of immune sytem and endothelial Cell. Tumor necrosis factor results in endothelial cell activation and it results in an enhanced expression of the adhesion molecules and chemokines[157]. The impact of tumor necrosis factor on angiogenic activity is dose dependent and also time reliant. It is also influenced by other tumor necrosis factor dependent mediators like vascular endothelial growth factor or platelet activating factor[157-159]

TNF stimulates ceratin proangiogenic mediators, such as vascular endothelial growth factor , interleukin-8 and basic fiboblast growth factor in endothelial cell[160] and these factors exerts both proangiogenic as well as antiangiogenic effects. Tumor necrosis factor was proved to inhibit endothelial cell proliferation *in vitro* initially, but it also causes new vessel formation in in vivo studies in the rabbit cornea micropocket assay [161]. TNF can be secreted by any type of cells. Mast cell stores preformed tumor necrosis factor, that is released in a rapid manner on

appropriate stimulation. Consequently increased levels tumor necrosis factor mRNA and its protein could be detected in psoriatic skin lesions [162]. Therapies that blocks the activity of tumor necrosis factor leads to clinical improvement of psoriatic skin lesions and also decrease in expression of the proangiogenic factors. Thus tumor necrosis factor takes part in angiogenesis which is associated with psoriatic skin lesions . It is still debatable whether TNF causes angiogenesis directly or indirectly through the stimulation of proinflammatory cytokines or angiogenic factors.

INTERLEUKIN 8(IL-8)

Interleukin 8 was isolated firstly from the scales of psoriasis[68]. Interleukin 8 or CXCL8 belongs to a group of chemokines - CXC, that is characterized by four cysteins which is highly conserved with first 2 cysteins separated by a nonconserved amino acid - CXC[163-165]. Interleukin 8 is a potential chemo attractant factor for neutrophils, Tlymphocyte and basophils. It is also plays a role in several auto immune diseases, inflammation and infections[165]. Interleukin 8 is stimulated by by Interleukin-1, TNF, Interleukin-6, Interferon -γ, lipopolysaccharide, reactive oxygen species and othercellular mediators . Interleukin-8 is a strong proangiogenic factor. The proangiogenic properties of interleukin -8 are independent of its proinflammatory functions, as interleukin-8 can

as well stimulate angiogenesis even when the inflammation is absent[167,168]. Interleukin -8 has also been shown to stimulate endothelial Cell proliferation, survival ,migration and expression of matrix metallo proteinases. Therefore, Interleukin-8 was shown to promote endothelial cell migration and endothelial cell proliferation and tube formation of endothelial cell *in vitro*[166,169,170]. Also, Interleukin -8 promotes endothelial cell survival by the inhibiting apoptosis of EC by inducing antiapoptotic proteins and down regulation of pro apoptotic proteins like Bax in endothelial cells [170]. It can induce the activity of matrix metallo proteinases 2 and 9. The *in vitro studies* demonstrates proangiogenic properties of interleukin-8 and established by various *in vivo* assays[166-168].

Several type of cells produce interleukin -8, which includes immune cells like mast cells , neutrophils or T cells[171], keratinocytes [122] and EC[172]. Consequently interleukin -8 is upregulated in psoriasis skin and downregulated after successful treatment[70]. Increased interleukin-8 and its receptor mRNA can be demonstrated within the epidermis of psoriasis skin lesions. Immunohistochemistry can localize Interleukin 8 protein to suprabasal keratinocytes and neutrophils[171,173,174]. As interleukin-8 stimulates proliferation of keratinocyte [175] and thus IL-8 stimulates the majority of cell types in psoriasis.

INTERLEUKIN 17(IL-17)

The proinflammatory cytokine interleukin 17 was termed formerly as cytotoxic T lymphocyte associated antigen 8 and now known as interleukin -17 A [176]. The Interleukin-17 cytokine is in a family of six members, interleukin-17A to F, which plays a role in inflammation and autoimmune diseases like psoriasis and cancer[177]. IL-17A also stimulates the secretion of chemokines, various growth factors and several adhesion molecules by epithelial cells, fibroblast and endothelial cells, which includes Interleukin-6, Interleukin-8, Interleukin-1, GM-CSF, G-CSF, and intercellular adhesion molecule 1. Therefore, interleukin-17 potentiates accumulation of neutrophils as well as granulopoiesis. Also , Interleukin -17A triggers the expression of tumor necrosis factor and interleukin 1β by the macrophages [178]. The stimulation and production of Interleukin -17A during CD 4 or CD 8 memory T cell differentiation is regulated by an array of intimately related cytokines, which includes Transforming growth factor β, Interleukin-6, Interleukin 21 and Interleukin -23.

IL-17A is a proangiogenic factor[91]. It can induce neovascularisation in the rat cornea micro pocket assays. Interleukin 17A over-expressing tumour cells induces a swift neoplastic growth with a significant increased tumour neovascularization *in vivo*[91]. *In vitro*, IL-

17A stimulates endothelial cell migration and cord formation. Interleukin 17A induces expression of proangiogenic mediators, which includes vascular endothelial growth factor that is responsible for the proangiogenic effects of Interleukin-17A.

Though an array of cytokines was found to be involved in pathomechanism of psoriasis, but these cytokines alone cannot be considered causing psoriasis.[179] The key cytokines involved in the pathobiology of psoriatic skin lesions are:

TUMOR NECROSIS FACTOR ALPHA- causes stimulation of epidermal keratinocytes to produce ICAM-1,IL-8, ,TGF ALPHA, BETA Defensins, GM CSF and plasminogen activator inhibitor 2. Increase the capacity of macrophage to secrete proinflammatory cytokines. It also stimulates endothelial cell to secrete VEGF and increases keratinocyte proliferation.

INTERFERON GAMMA – it induces intercellular adhesion molecule 1 expression on epidermal keratinocyte and vascular endothelial cell and thus influences the migration of lymphocytes into lesional skin. It also stimulates APC activity and TNF aipha release from phagocytes.

GM-CSF – It increases keratinocyte proliferation, activation of neutrophils,stimulates migration and proliferation of endothelial cells

INTERLEUKIN-1 – induces expression of E SELECTIN,ICAM 1,VCAM 1 on epidermal keratinocytes and expression of keratinocyte growth factor and GM-CSF on fibroblasts which promotes keratinocyte proliferation and differentiation.

INTERLEUKIN-2 – It is a growth factor and chemoattractant for T cells as well. It also stimulates NK cell activity and induces T cell cytotoxicity.

INTERLEUKIN 6 – increase the proliferation, activation and chemotaxis of T lymphocyte in dermis. Activation and proliferation of B lymphocytes and macrophages.

INTERLEUKIN 8- migration of Tcells and neutrophils to the epidermis. It also activates and helps in proliferation of Tlymphocytes along with stimulation of angiogenesis

INTERLEUKIN 12- stimulates type 1 Tcell maturation pathway

EPIDERMAL GROWTH FACTOR family – increased EGF/TGF alpha receptors in psoriatic epidermis

VASCULAR ENDOTHELIAL GROWTH FACTOR - it is icreased in psoriasis and causes erythema. Plays a role in regulation of vascular growth and vascular remodelling in psoriasis. It acts as alink between angiogenesis and inflammation in psoriasis.

NERVE GROWTH FACTOR- overexpressed in psoriasis. It stimulates keratinocyte proliferation,endothelial cell proliferation and adhesion molecules expression.

INTERLEUKIN 23- It increases the levels of Interleukin-17 and Interleukin-22. It induces T helper17 cells and activates nuclear STAT3 transcription. It causes acanthosis and mixed inflammatory infiltration.

INTERLEUKIN 22- In association with IL-17 ,it induces defensins,MMP and molecules like s100A7 which induces keratinocyte mobility.

INTERLEUKIN- 17- increases the expression of ICAM-1 on fibroblast.

Recent studies has identified a chemokine CX3C L1 known as Fractalkine in Psoriasis in human beings. CX3C L1 is produced in the form of a lengthy protein with 373-amino acid with an extended mucin like stalk with a chemokine domain on the top. The mucin like stalk allows fractalkine to attach to the surface of several types of cells. But a soluble form of this chemokine has been demonstrated. Soluble form CX3C L1 strongly chemoattracts T lymphocytes and monocyte, whereas the cellbound form of chemokine enhances adhesion of leukocyte to the stimulated ECs. Fractalkine binds to its seven trans-membrane domain G protein coupled receptor CX3C R1 and this binding induces signalling and mediates cellular adhesion.[180,181] CX3C L1 is also expressed in several organs such as brain, heart, kidney,heart, muscle,lung and testis

wherein it interacts with a single G Protein Coupled Receptor and CX3CR1 induces chemotaxis and adherence of CX3C R1 expressing cells which includes neutrophils, Th-1 cells,natural killer cells and monocytes. [182]

SUMMARY OF PATHOGENESIS OF PSORIASIS

The inflammatory infiltrate seems to be the prime reason for the whole pathogenesis of psoriasis. There is a significant correlation of epidermal hyperplasia with the inflammatory infiltrate, spongiform pustules of Kogoj, capillary proliferation and parakeratoses .

Studies have shown that aberrant regulation of T lymphocytes along with interaction between epidermal keratinocytes and a composite array of cytokines are involved in the pathomechanism of psoriasis. An injury to these defective epidermal keratinocytes results in activation of synthesis and release of several cytokines. These cytokines enhances T lymphocyte activation.This leads to secretion of several cytokines and various growth factors by the T cells, in addition to further proliferation of keratinocytes and thus a vicious cycle of events. This cycle explains there is a significant correlation between the degree of epidermal hyperplasia and the inflammatory infiltrate. One school of thought is that neutrophils are recruited by the neutrophil-attracting chemokine interleukin-8 (CXCL8). IL 8 mRNA is can be synthesized by the CD 4

subset of T cells. There appears to be a strong correlation between the inflammatory infiltrate and the grade of capillary proliferation and also a significant correlation between the epidermal hyperplasia and the grade of capillary proliferation ._ It is also believed that Vascular Endothelial Growth Factor and IL -8 released from epidermal keratinocytes contributes to the neovascularization in psoriasis. Several studies suggests that lymphocytes releases angiogenic factors which induces capillary proliferation and vasodilatation[7]. A series of experiments by Mor *et al*., proved that T cells can synthesize and secrete VEGF[183]. Immune processes and inflammatory cascades are well known inducers of angiogenesis and also angiogenesis promotes and maintains immune responses and inflammatory cascades[98]

HISTOPATHOLOGICAL FEATURES OF PSORIASIS

Psoriasis is a dynamic process and hence the histopathological features varies during the evolution and subsequent resolution of individual lesions.

EARLY LESIONS

The earliest change, seen in lesions of less than 24 hours' duration, consists of dilatation and congestion of blood vessels in thepapillary dermis with a mild, perivascular, lymphocytic infiltrate, and adjacent edema. There is also some exocytosis of lymphocytes into the epidermis

overlying the vessels and this is usually associated with mild spongiosis . The epidermis is normal. This is soon followed by the formation of mounds of parakeratosis, with migration of neutrophils through the epidermis to reach the peaks of these parakeratotic . There is overlying orthokeratosisof normal basket-weave type and loss of the underlying granular layer[4].

PAPULAR LESIONS

At the papular stage, there is increased mitotic activity can be seen in the basal layer of the epidermis associated with a psoriasiform acanthosis . Keratinocytes in the upper epidermis shows cytoplasmic pallor. Blood vessels in the papillary dermis are dilated and somewhat tortuous, and their lumen might contain neutrophils. Lymphatic channels are also increased. Few neutrophils are ever present in the perivascular infiltrate: this consists of lymphocytes, Langerhans cells, and indeterminate cells. A few extravasated erythrocytes may also be seen. These changes are also seen in guttate psoriasis although the mild epidermal hyperplasia is usually seen in this variant of psoriasis.

PLAQUE LESIONS (EARLY AND LATE LESIONS)

In early plaques of psoriasis and in 'hot spots' of established plaques lesions, there is of parakeratosis containing neutrophils, which migrates to the upper layers of mounds of parakeratosis. With time, confluent parakeratosis is seen . Several layers of parakeratosis

containing neutrophils, with intervening layers of orthokeratosis, are present. Intracorneal collections of neutrophils known as Munro microabscesses are common, similar collections in the spinous layer known as spongiform pustules of Kogoj are less common. They are also much smaller than in pustular psoriasis[4]. These pustules contain lymphocytes in addition to neutrophils. The epidermis shows psoriasiform (regular) hyperplasia, with suprapapillary plate thinning overlying the dilated vessels of the papillary dermis . Increased expression of Ki 67 is noted . A few mononuclear cells are usually present in the lower layers of the suprapapillary epidermis.

The dermal inflammatory cell infiltrate is usually a heavier than in earlier lesions. The dermal inflammatory infiltrate includes activated T lymphocytes fewer Langerhans cells as compared to earlier lesions, and occasional neutrophils. A subset of spindle-shaped macrophages are situated along the basement membrane, and it is described as a characteristic feature.

They are known as 'lining cells' that are positive for CD11c[4]. Plasma cells and eosinophils are usually absent, but eosinophil cationic protein is identified, particularly in the upper third of the epidermis in psoriastic lesions.

Plasma cells may be present in patients with HIV infection.With time, there is club-shaped thickening of the lower rete pegs with

coalescence of rete pegs in some areas. Later lesion shows orthokeratosis, an intact granular layer, and thickening of the suprapapillary plates. Migration of inflammatory cells is usually mild. The finding of many fatty vacuoles in the papillary dermis – pseudolipomatosis cutis is of less significance.

Differentiation of late plaque lesions of psoriasis from lichen simplex chronicus might be difficult, but in contrast in lichen simplex chronicus , the suprapapillary plates and granular layer are more prominent and vertically oriented collagen bundles are seen in the dermis. The histopathological features of the psoriasis lesions may be obscured by superimposed changes due to rubbing or scratching

PSORIATIC NEURODERMATITIS

The terminology psoriatic neurodermatitis is used for pruritic, lichenified plaques seen on the elbows and/or knees[4]. Lesions are numerous in number, smaller in size , more keratotic, and less excoriated than lichen simplex chronicus.

HISTOPATHOLOGY OF PSORIATIC NEURODERMATITIS

Microscopically the lesions show microabscesses in the horny cell layer, regular acanthosis, hypogranulosis and suprapapillary plates

thinning in epidermis. One school of thought is that these cases represent psoriasis with superimposed lichen simplex chronicus.

TREATED PLAQUE LESIONS OF PSORIASIS

In resolving or treated plaques of psoriasis there is a marked decrease in the inflammatory infiltrate, a decrease in the amount of hyperproliferation of epidermal keratinocytes, and normalisation of the granular cell layer. Vessels in the papillary dermis are dilated, albeit by this stage there is an increase in number of fibroblasts in this region with only mild fibrosis. After a period of 10–14 weeks of treatment , the histological appearances return to normal.

HISTOPATHOLOGICAL CHANGES IN SCALP REGION

Changes reported in psoriasis of the scalp are minor changes only and includes sebaceous gland atrophy, a decrease in size of hair follicle and thinner hair shafts. Other features of scalp psoriasis includes infundibula dilatation with parakeratosis at the lips of the ostium of infundibula, papillomatosis, and scattered apoptotic keratinocytes. Munro microabscesses are not common in this region.

Regional variations includes reduced epidermal hyperplasia seen in psoriatic lesions of the penis and vulva; spongiosis may be present.

Spongiosis is a feature of the early lesions of psoriasis, and of psoriasis occurring in various regions such as the hands and feet and genital regions. It may also occur in erythrodermic psoriasis . The

terminology *spongiotic psoriasis* is given for those cases with significant spongiosis in early stages , but with time evolves into classic psoriasis. The initial biopsies show spongiosis, mounds of parakeratosis containing neutrophils, dilated vessels in the papillary dermis, and a mild, superficial perivascular infiltrate of lymphocytes.

NAIL CHANGES IN PSORIASIS

The nail plate in nail lesions of psoriasis shows hyperkeratosis, focal parakeratosis, and variable number of neutrophil exocytosis into the parakeratotic layer. Spongiosis is one of the common features of nail psoriasis. Examination of periodic acid Schiff 's stained sections is required before making a diagnosis of nail psoriasis because of its similar histological features to onychomycosis.

HISTOPATHOLOGIC FEATURES OF ERYTHRODERMIC PSORIASIS

The histopathologic of erythrodermic psoriasis appearances resembles those seen in early lesions of psoriasis, possibly a reflection of the early medical treatment that is usually given in this condition. Dilated superficial vessels is usually prominent. A cornified layer is absent. Sometimes the histological features might not resemble those of psoriasis at all.

HISTOPATHOLOGICAL FEATURES OF FOLLICULAR PSORIASIS

The histopathological features in follicular psoriasis are follicular plugging with marked parakeratosis in the mid-zone of the ostium. Both perivascular and perifollicular dermal inflammatory infiltrate is noted.

HISTOPATHOLOGICAL FEATURES OF ANNULAR VERRUCOUS PSORIASIS

The features are exaggerated papillomatosis resulting in finger-like projections of the epidermis. The papillomatosis and bowing of the peripheral rete ridges toward the center of the psoriatic lesion mimics the appearance of verruca vulgaris.

HISTOPATHOLOGY OF GENERALISED PUSTULAR PSORIASIS

Macro Spongiform pustules of kogoj are seen in all types of variants of generalised pustular psoriasis and it is representative of characteristic histopathological lesion. The spongiform pustular is formed due to migration and accumulation of neutrophils from papillary dermal vessels to the uppermost layers of epidermis. The neutrophils accumulates within the interstitium of sponge like network formed by

degenerated and degraded epidermal keratinocyte. In a large pustule, the epidermal keratinocyte in the middle of the lesion undergoes cytological destruction resulting in formation of a large cavity, however in the periphery of the lesion the thinned out epidermis persists. When the neutrophils in the pustular lesion migrates up into the horny layer, they become pyknotic forming a large munro abscess. Other features are parakeratosis, elongation of rete ridges. Dermis reveals lymphocytic infiltrate[4].

HISTOPATHOLOGY OF LOCALISED PUSTULAR TYPE OF PSORIASIS

Early lesion shows spongiosis and extravasation of lymphocytes in the lower epidermis overlying papillae of dermis. This is followed by formation of small intra epidermal vesicle which contains only lymphocytes . There is massive extravasation of neutrophils which penetrates the intercellular spaces in the wall of the vesicular lesion, forming spongiform pustules. In the acute type, pustular bacterid, leukocytoclastic vasculitis is noted.

DIFFERENTIAL DIAGNOSIS OF PSORIASIS

1. Mycosis fungoides or cutaneous Tcell lymphoma
2. Pityriasis rosea

3. Nummular dermatitis
4. Pityriasis lichenoides chronica
5. Pityriasis rubra pilaris
6. Secondary syphilis
7. Bowen's disease
8. Acute generalized exanthematous pustulosis
9. Hypertrophic lichen planus
10. Sneddon–Wilkinson disease
11. Small plaque parapsoriasis
12. Intertrigo
13. Langerhans cell histiocytosis
14. Dyshidrotic dermatitis
15. Tinea manuum/pedum/capitis
16. Seborrheic dermatitis

Chronic plaque type of psoriasis should be differentiated from mycosis fungoides type of cutaneous T cell lymphoma. Mycosis fungoides shows signs of atrophy of epidermis or poikiloderma which is a differentiating feature from plaque type psoriasis; but a biopsy of skin is required to differentiate between the two[184].

Pityriasis rubra pilaris- PRP is differentiated from plaque type psoriasis clinically by the occurrence of reddish-orange palm plantar

keratoderma, keratotic follicular papules and classically spares the trunk. Histopathologic features are alternate horizontal , vertical orthokeratosis and parakeratosis with follicular plugging that helps to differentiates PRP from psoriasis.

Bowen's disease and Nummular dermatitis are differential diagnosis of plaque type psoriatic lesions.

Nummular dermatitis is more pruritic than psoriasis and linked to previous history of atopy. Histological study is more dependable to distinguish nummular dermatitis from psoriasis plaque and Bowen's disease. If psoriasis occurs in the tibial shin, hypertrophic lichen planus should be thought of, however a typical lichen planus lesion occurs elsewhere on the body in addition to involvement of mucosa helps to distinguish between the two[184].

Guttate psoriasis can be diagnosed in a staright forward manner, but the differential diagnosis includes small plaque type para-psoriasis, pityriasis rosea, secondary syphilis and pityriasis lichenoides chronica clinically.These entities should be differentiated from guttate psoriasis based on history , clinical findings , histopathologic features, and laboratory values.

Small plaque type parapsoriasis presents with various types of erythematous plaques and roofed by fine scales.Infrequently, it might present with lengthened, finger like patches that are symmetrically dispersed on the flanks, and it is called as “digitate dermatosis.”

Pityriasis rosea is differentiated from psoriasis by the presence of herald patch and remission of the disease in a duration of few months.

Secondary syphilis should be clinically differentiated based on palmoplantar involvement, that is not seen in guttate psoriasis, and on the basis of histopathological features and serological studies.

Pityriasis lichenoides chonica is characterized by clinical appearance of recurring crops of spontaneously regressing, reddish-brown papules with scales. Histopathologically, there is an interface dermatitis consisting of monoclonal T lymphocytic population predominantly and keratinocytes which are necrotic[184].

Pustular psoriasis resembles a pustule type of drug reactions, similar to acute generalized exanthematous pustulosis, SneddonWilkinson disease and Ig A pemphigus. The presence of peripheral eosinophilia and tissue eosinophilic infiltration on the histological section and a previous history medication intake favors the diagnosis of acute generalised exanthematous pustulosis.

SneddonWilkinson disease can be clinically differentiated by its annular type of plaques with flexor surfaces being more prone for lesions. The diagnosis of Ig A pemphigus can be given by using direct immunofluorescence studies which is positive,and is absent in both psoriatic skin lesion and SneddonWilkinson disease. The annular type of pustular psoriasis mimics SneddonWilkinson disease.

The important differential diagnosis of inverse psoriasis includes intertrignous lesions and if infants- Langerhans cell histiocytosis(LCH) . Patients with LCH can have scales and crusts on the scalp, as well as affecting internal organs such as liver enlargement , lytic bony lesions. Histopathological examination of skin should be diagnostic.

The differential diagnostic consideration for palmoplantar type of psoriasis includes dyshidrotic dermatitis and tinea manuum or pedum. Yellowishbrown macular lesions mixed with sterile pustular lesions favors palmoplantar type of psoriasis. The KOH preparation of scales helps to diagnose a dermatophytic infection[184].

Nail psoriasis mimics lichen planus, alopecia areata or trachyonychia. Pitting of nails is a common feature for both alopecia areata and Psoriasis.

Nail pits are larger, deeper and distributed in an irregular manner in psoriasis whereas in alopecia areata nail pits are smaller in size, superficial and distributed in a regular manner. Oil drops, splinter hemorrhages and distal onycholysis helps to differentiate between the two disorders. Thinning of nails laterally , linear ridges , fissures and dorsal pterygium are some of the features that favors the diagnosis of lichen planus. Trachyonychia or also known as twenty nail dystrophy can be owing to psoriasis, alopecia areata, or lichen planus,. In case of lack of cutaneous features, biopsy of nails helps to arrive at a diagnosis. Scalp psoriasis resembles tinea capitis and seborrheic dermatitis. Psoriasis could be clinically differentiated from tinea capitis, laboratory tests are required to confirm the diagnosis of dermatophytosis.

Laboratory tests includes a KOH examination, microbiological culture studies for fungi or histopathological examination of a skin biopsy .

Seborrheic dermatitis and psoriatic lesions on the scalp presents clinically in a similar manner and responds to the similar topical treatment. However the occurrence of psoriatic plaque lesions on the trunk along with a positive family history of psoriasis favors diagnosis of psoriasis.

Histopathological examination is the gold standard for distinguishing between seborrheic dermatitis and psoriasis. Lastly psoriatic erythroderma has a larger differential diagnosis, and there are several etiologies for erythrodermic psoriatic lesions which includes seborrheic dermatitis, Sezary syndrome ,atopic dermatitis,pityriais rubra pilaris, drug reactions, and graft versus host disease. A skin biopsy establishes the underlying etiology. Classic type of psoriatic plaques precedes psoriatic erythroderma but typical psoriatic features are lost when there is development of generalized erythema. Nail changes like pitting of nails , oil drop and onycholysis might be seen and provides a hint to the diagnosis of psoriasis erythroderma[184].

CLINICAL FEATURES OF PSORIASIS

Psoriasis is a papulosquamous disorder with varied morphologic features, different distribution , severity, and variable course. It classically presents as circular and well circumscribed erythematous papular lesions or plaque leions with a greyish or silverywhite dry scales. The psoriatic lesions are symmetrically dispersed on the scalp body fold region, elbow, knee and lumbosacral area,. Psoriasis can develops at the sites of physical damage or chemical damage which is known as Koebner phenomenon. Uncontrolled psoriasis leads to condition known as generalised exfoliative

erythroderma. Involvement of nails can be present, especially when psoriatic arthritis is present.

Psoriatic lesions might occur in the oral mucosal region or tongue. If it involves the dorsal surface of tongue , lesions will have sharp circumscribed gyrate reddish patch with a whitish yellow border. The patches might extend , changing on a regular basis, forms specific annular pattern and resembles a map, so it known as *geographic tongue*.

Psoriasis has a varied morphologic features, distribution, and intensity . Albeit the classic clinical presentation as above, the morphology can ranges from smaller papules- guttate psoriasis to pustular lesions -pustular psoriasis and generalised erythematous lesions with scaling – psoriatic erythroderma. Also these different types of psoriasis might be limited to small area or generalised. Psoriasis has a varied course presenting as chronic plaque or presents in an acute form that progresses rapidly with widespread involvement[185]. Psoriasis can be symptomatic with complaints of severe itching or burning sensation.

Several studies in India have studied the varied clinical features of the clinical disease in psoriatic patients. Okhandiar *et al*.[186] collected epidemiological data of 116 patients with psoriasis from several medical institutions. They observed that the extensor aspect (93%) is the more common site of occurrence followed by the scalp region (88%). Face,

palms, soles and nails are also involved in one third of the psoriasis patients. Flexural psoriasis was not common. None of their patients had oral mucosa affected.

Bedi [187] analyzed data of 530 cases of psoriasis for duration of 5 years . Chronic plaque psoriasis seemed to be the more common (90%) clinical presentation. The general sites involved are in a descending order are trunk, limb, scalp region, face, palmar areas ,soles and flexural region. The second common clinical presentation is palmo plantar type of psoriasis followed by inverse psoriasis. He also found that guttate psoriasis, oral mucosa affection, and erythrodermic psoriasis are rare.

Kaur *et al*. [188] found that scalp region (25%) as the more common site of involvement followed by lower limbs (20.6%) and upper limbs (11.7%). Oral mucosal (0.7%) and genital (0.4%) mucosal involvement is not common. Chronic plaque psoriasis (93%) was the more common clinical presentation. Palmo plantar pustular psoriasis , guttate psoriasis and erythrodermic psoriasis accounted for less than 2% of cases for each variant. Generalized pustular psoriasis, isolated nail psoriasis, flexural psoriasis and psoriatic arthritis were not so common.

The various clinical presentation of psoriasis are given below[185]

PLAQUE TYPE OF PSORIASIS (PSORIASIS VULGARIS)

The most common form of psoriasis is plaque type psoriasis which presents as a well circumscribed, round to oval, or nummular plaques . The lesions first begins as an erythematous flat macules less than one cm or papules, which spreads to the peripheral areas and coalesces to result in plaque lesions of 1cm to many centimetres in diameter. A whitish blanched ring also called as Woronoff's ring, is seen in the skin around a psoriasis plaque. With further spread of lesions to the periphery , plaque develops different configurations which includes:

- psoriasis gyrate - curved linear type are seen predominantly
- annular type of psoriasis - ring like lesions that develops secondary to central clearing
- psoriasis follicularis - minute scaly papules located at the opening of
- pilo-sebaceous unit.

The rupioid type of psoriasis and the ostraceous type refers to specific morphologic subtypes of plaque type of psoriasis.

- Rupioid lesions are smaller in size with two to five centimetres in diameter and markedly hyperkeratotic lesions which resembles limpet shells.
- Ostraceous subtype are hyperkeratotic plaque with a concave centre, simulating an oyster shell.[185]

Scales are present in plaque type of psoriasis, which characteristically has silvery whitish appearance, and varies in thickness. Scraping of scales reveals smaller bleeding points known as Auspitz sign. The quantity of scales vary among different patients and also at different sites of lesions in the same patient. The acute inflammatory or exanthematic psoriasis, scaling is minimal and erythematous lesions might be the prominent clinical feature.

In cutaneous involvement, plaque type of psoriasis is associated with internal involvement, which includes joints and extraarticular sites like the eyes. Coexistent psoriatic arthritis is present in 5%–30% of psoriatic patients .[189] In a minority of patients, the features of psoriatic arthritis appears before affecting the skin. The classical clinical manifestation is asymmetrical oligo-arthritis with involvement of the distal and proximal interphalangeal joints of the hand and foot. Erosive change occurs years after the presenting peri-articular inflammation. The occurence of eye involvement in patients with psoriasis is yet to be known; but occurs in approximately 10% of psoriasis cases.[190] Psoriasis can involve almost any part of the eye which results blepharitis, cataract formation, peripheral keratopathy, acute anterior uveitis, , conjunctivitis and posterior synechiae.

GUTTATE TYPE OF PSORIASIS

Guttate type of psoriasis, comes from the Greek word *gutta* which means a droplet, and it has sudden onset of small,two to ten millimetre diameter lesions of psoriasis. The lesions are distributed in a centripetal manner albeit guttate lesion also involves the head, arms and legs. Typically, the guttate psoriasis presents following an acute group beta haemolytic streptococci infection of the pharyngeal or tonsillar region and might be the first clinical presentation of psoriasis in children or in adults which is rare. The number of lesion ranges from five or ten to hundred . Guttate psoriasis is accountable for two percent of the total number of psoriasis cases. In children, the acute form of guttate psoriasis is self limiting illness; whereas in adults, acute guttate form complicates long standing psoriatic plaques. A small study showed that 33% of patients with acute guttate psoriasis finally develop into chronic plaque psoriasis.[191]

INVERSE(FLEXURAL) PSORIASIS

Psoriasis which affects the flexural areas such as inframammary region, perineal area, and axillary region, has different morphological feature when compared to usual type of plaques occurring somewhere else on the trunk ,upper limb and lower limb. Flexures with plaques do not have scales and appears as reddish, shiny, well circumscribed

plaques sometimes confused with candida intertrignous infection, and dermatophyte infection.

ERYTHRODERMIC PSORIASIS

Complete or subtotal involvement of the skin by active psoriatic lesions is called as erythrodermic psoriasis . First is that chronic plaque psoriasis gradually progresses , that becomes continous and widespread. Second is that erythroderma might be a clinical presentation of unstable type of psoriasis which is triggered by infections, tar, drug intake, or withdrawal of steroids. Erythroderma affects the temperature-regulatory efficiency of the skin which results in decrease in body temprature, high output heart failure, and several metabolic changes like hypo-albuminaemia, and severe anaemia due to loss of iron, vitamin B_{12} and folic acid.

GENERALISED PUSTULAR TYPE OF PSORIASIS

Generalised pustular type of psoriasis -von Zumbusch is very rare and it represents an unstable active form of psoriasis. Systemic or powerful topical steroids and infection precipitates this clinical subtype. The patient will have fever, with reddish, painful, inflammed skin studded with uniform looking, sterile pustular lesions, that coalesces to form larger sheet of lesion. Patients with generalised pustular type of psoriasis needs admission in the hospital for further treatment.

PALMOPLANTAR TYPE OF PUSTULOSIS

Palmoplantar pustular lesion manifests as sterile, yellowish pustular lesions on an erythematous background with scaling , that affects the palmar areas and soles . The pustular lesions are painful and fades away to form dark brownish colouration with sticky scales or crusts formation. Palmo plantar pustulosis also have affected nails. Around 25% of patients are related to classic type psoriasis vulgaris, but it is now thought that palmoplantar pustulosis might not be a type of psoritic lesion.[192] This assumption was derived from genetic studies that showed no link with HLA-Cw6 or other genetic markers on chromosome 6p that are associated with chronic plaque type psoriasis and guttate type of psoriasis. The epidemiology of palmoplantar pustulosis is entirely different from chronic plaque type psoriasis in that it affects women most commonly , mostly presents in the age group of 40 to 60 years, and has a strong association with smoking, which may be either present smoking or past history of smoking, in 95% of patients.[193]

PSORIATIC NAIL DISEASE

Finger nails are mostly affected than the toe nails. The common findings are tiny pits in the nail plate, that results from deficient nail formation in the proximal part of the nail matrix . The nail detaches

from the nail bed at its distal attachment or its lateral attachment ,which is known as onycholysis . Orangish yellow areas might be seen below the nail plate ares and are known as “oil spots”. The nail plate becomes thickened , also dystrophic and discolored nail plate is also noted. Yellowish keratinous material might get collected beneath the nail plate and it is termed as subungual hyperkeratosis.

OTHER DISEASE ASSOCIATION WITH PSORIASIS

Patients with psoriatic lesions are at risk of several other diseases due to sharing of genetic pathway , similar immune cascades, therapy associated adverse effects and toxicities, and allied psychological stress of the disease[184].

Psoriatic patients have an higher incidence of inflammatory bowel disease specifically Crohn’s disease, that might be owing to sharing of genetic pathways. Other diseases associated are obesity, metabolic syndrome, type 11 diabetes, malignancy and psychological co – morbidities. Epidemiological studies shows that patients with psoriatic skin lesions have a increased risk of developing specific metabolic disorders like obesity. Mechanism by which psoriasis leads to obesity has been suggested, includes higher incidence social isolation, unhealthy diet, increasing depression, intake of alcohol and decrease in physical exercises. Few studies demonstrates that a increased occurrence of

diabetes in psoriasis independent of usual risk factors for such as age, gender, obesity, hyperlipidemia and hypertension[184].

HIV associated psoriasis occurs with an increased occurence than in general population. Psoriasis can also present for the first time in a later stages of retroviral infection, when the CD 4 cell count is less than 100 cells/ microliter . Psoriasis also spontaneously improves in the end-stage AIDS[184].

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Materials and Methods

MATERIALS AND METHODS

STUDY DESIGN

This is a case control study carried over a period of 15 months from April 2013 to July 2014 in Coimbatore Medical College and Hospital.

PATIENT SELECTION

Thirty two psoriasis cases selected from Department of Dermatology in Coimbatore Medical College. Patients included are newly diagnosed psoriasis cases and patients did not receive any topical or systemic treatment for psoriasis for at least two months prior to the study. Skin biopsies are taken from psoriasis patients attending dermatology department in Coimbatore College From every patient, elliptical biopsy is taken from skin unexposed to sunlight involving lesional skin. Controls were from skin such as circumcision of healthy persons and normal skin from other departments such as plastic surgery.

Patients with psoriasis vulgaris are assessed for severity of lesions by a scoring system known as PASI score. It is based on lesions, area of distribution of lesions. Finally distribution of lesions for the entire whole body is calculated.

TABLE - 1

PASI SCORE- SEVERITY OF LESION

INDURATION, ERYTHEMA, SCALING	**No symptoms**	**Slight**	**Moderate**	**Marked**	**Very marked**
SCORE	0	1	2	3	4

TABLE - 2

PASI SCORE –AREA OF DISTRIBUTION

AREA	**0**	**1-9%**	**10-29%**	**30-49%**	**50-69%**	**70-89%**	**90-100%**
SCORE	0	1	2	3	4	5	6

TABLE - 3

PASI SCORE – METHOD OF CALCULATION

SCORE FOR THE LESION	HEAD	TRUNK	UPPER EXTREMITIES	LOWER EXTREMITIES & BUTTOCK
INDURATION				
ERYTHEMA				
SCALING				
SUM=I+E+S				
% OF AREA AFFECTED				
AREA SCORE				
SUBTOTAL= AREA SCORE X SUM				
BODY AREA= SUBTOTAL X AMOUNT	**X 0.1**	**X 0.3**	**X 0.2**	**X 0.4**
TOTALS				

PASI SCORE = HEAD + TRUNK + UPPER LIMB + LOWER LIMB

PASI SCORE

PSORIASIS AREA SEVERITY INDEX is a useful and important tool to calculate the severity of the disease and does not have specific cut off values. It is based on site of distribution , morphology of lesions and percentage of area of distribution[193].

INCLUSION CRITERIA

Adults with newly diagnosed Psoriatic skin lesions with age 20 to 65 years.

EXCLUSION CRITERIA

Patients already diagnosed and undergoing topical or systemic therapy psoriasis for less than two months prior to the study.

The biopsy was cut into two portions. Tissue is formalin fixed and subjected to routine tissue processing. This is followed by haematoxylin and eosin staining and evaluated by light microscopy. Psoriasis was diagnosed by clinical features and confirmed by histology.

HAEMATOXYLIN AND EOSIN STAINING FOR ROUTINE HISTOLOGIC SECTIONS

All specimens are fixed in 10%formalin , subjected to routine tissue processing and 4-6 μm sections were cut on glass slides for routine Haematoxylin and Eosin staining.

REAGENTS

1. Erhlich's Haematoxylin solution
2. Eosin Y solution 1%
3. 1% acid alcohol solution

PROCEDURE

1. Sections are deparaffinised.
2. Dip the sections in xylene for atleast 30 seconds.
3. Place the section in isopropyl alcohol for fifteen minutes.
4. Then the sections are washed under tap water.
5. Sections are stained with Erhlich's Haematoxylin for ten to fifteen minutes.
6. Again sections are washed under tap water.
7. Differentiation of sections in 1% acid alcohol solution by dipping the slide twice or thrice.
8. Slide is kept for blueing for ten minutes.

9. Counterstaining with 1% eosin solution by dipping the slide twice or thrice followed by tap water wash.
10. Sections are to be air dried.
11. Dip in xylene and mount in DPX.

The routine hematoxylin and eosin stained sections are studied under Olympus light microscope.and diagnosis of psoriasis was obtained by analysing histopathological features.

IMMUNOHISTOCHEMISTRY

This is a two step indirect technique based on detection of antigens in the cells and tissues. It is a two step process:

1. Specific epitopes binds the primary antibody
2. The following step is calorimetric reaction to detect the antigen antibody binding.

REAGENTS IN IMMUNOHISTOCHEMISTRY

1. Peroxide block which is 3% hydrogen peroxide in water
2. Power block : Is a highly effective universal protein blocking agent. It consists of casein and propriety additives in PBS with 15Mm sodium azide.
3. Chromogen : DAB – 3,3'- diaminobenzidine

4. Liquid DAB substrate buffer contains peroxide and stabilizers
5. Superenhancer reagent
6. Poly HRP reagent
7. Mayer's haematoxylin is used for counterstaining
8. Buffer solutions.

BUFFER SOLUTIONS

TRIS BUFFER (pH 7.6)

TRIS buffer salt : 0.605 gm

Sodium chloride : 8gm

Distilled water : 1000 ml

1N Hydrochloric acid : 3ml

CITRATE BUFFER : (pH 6.0)

Trisodium citrate : 2.94gm

Distilled water : 1000ml

1N Hydrochloric acid : 5ml

TRIS EDTA : (pH 9.0)

TRIS buffer salt : 6.05gm

Disodium EDTA : 0.744gm

Distilled water : 1000ml

CHART – 1 : PRIMARY ANTIBODY WITH SECONDARY KIT

PROCEDURE OF IMMUNOHISTOCHEMISTRY

1. Sections are deparaffinised in xylene for 30 minutes.
2. Sections are washed in absolute alcohol for five minutes with two changes followed by tap water wash for ten minutes.
3. Sections are rinsed in distilled water for five minutes.
4. Antigen retrieval is done by immersing slides in appropriate buffer solutions in microwave – medium mode for fifteen minutes and high mode 10 minutes.
5. Cool to room temperature and then the slides are washed in distilled water.

6. The slides are washed in TBS buffer for five minutes with two changes. Sections are treated with peroxide block for ten minutes followed by wash in TBS buffer for five minutes with two changes.
7. Treat with power block for ten minutes.
8. Then drain the slides and cover the sections with primary antibody (supplied by biogenex) for one hour.
9. This is followed by TBS buffer wash for five minutes with two changes. Cover the sections with superenhancer for 30 minutes.
10. Wash in TBS buffer for five minutes with two changes. Cover the sections with poly HRP reagent for thirty minutes.
11. Then again wash the slides with TBS buffer for five minutes with two changes.
12. Sections are treated with DAB chromogen with substrate buffer for five to eight minutes.
13. Wash in TBS buffer with two changes, each wash for five minutes. Wash the slides under tap water for five minutes.
14. Counterstain the slides using Mayer's Haematoxylin for one minute. Wash under tap water for five minutes.
15. Slides are air dried and mount with cover slips using DPX mountant.

IMMUNOHISTOCHEMICAL EVALUATION

The slides are examined under Olympus light microscope. The degree of immunohistochemical reactivity for VEGF and VWFr are evaluated based on the level of staining of epidermis. They are divided into three groups :

(I) basal layer only - 1+

(II) lower half of the epidermis- 2+

(III) whole epidermis - 3+ .

VEGF and VWFr shows cytoplasmic staining.

The evaluation of staining in CD34 - microvessel densit y (MVD) was done by capillary counting method in the 3 highly vascularized areas selected also known as hot spots, under 40x field.CD 34 showed membranous and cytoplasmic staining. A 400x field was used to count microvessels in each of the highly vascularised areas. Single endothelial cell or clusters of endothelial cells, either with or without lumen, were considered to be individual vessels. The score is as follows:

(I) Mild (4-10 capillaries)

(II) moderate (11-20 capillaries)

(III) severe (21-28 capillaries).

Observation and Results

OBSERVATION AND RESULTS

The present study was a prospective study conducted in the department of pathology during the period of April 2013 to July 2014. Ethical clearance was obtained from the Ethics committee of Coimbatore medical college, Coimbatore.

A total of 62 samples were obtained and studied which included 32 cases of psoriasis vulgaris and 30 controls which is a normal healthy skin. Histopathological and immunohistochemical evaluation was done. Data was obtained , coded and entered into Microsoft excel spread sheet and analysed as below.

TABLE - 4

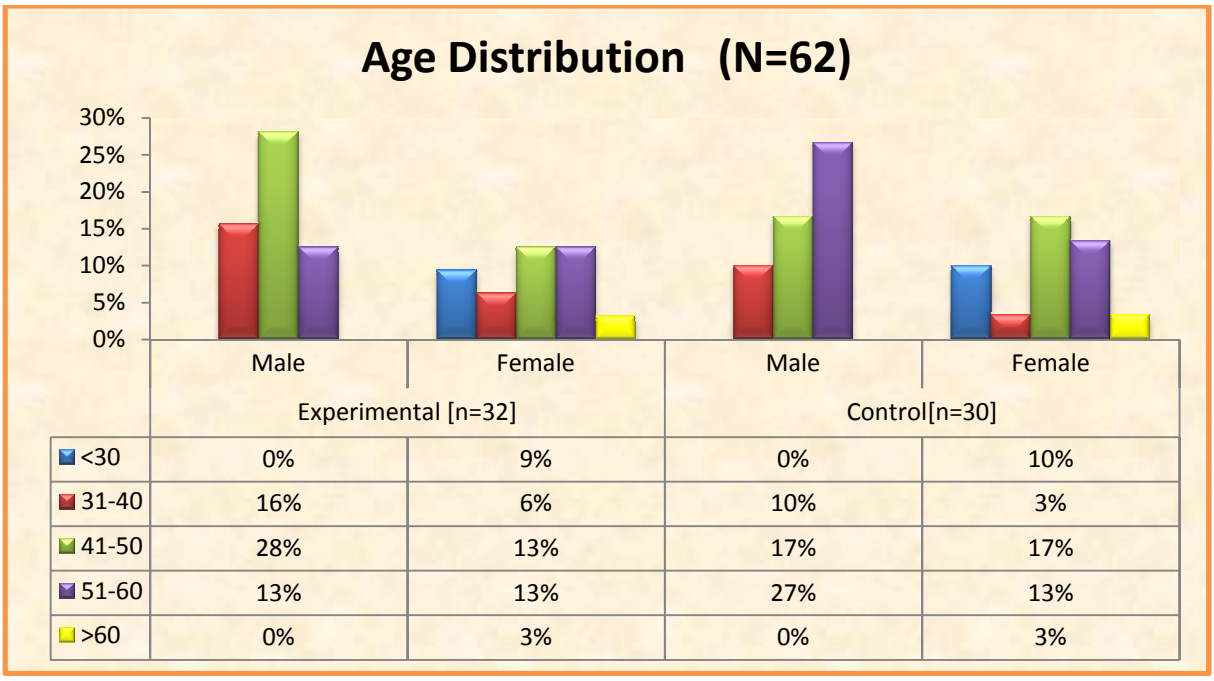
AGE WISE DISTRIBUTION OF PSORIASIS

AGE DISTRIBUTION n= 62					
AGE YEARS	**CASES**		**CONTROL**		**TOTAL**
	MALE	**FEMALE**	**MALE**	**FEMALE**	
<30	0	3	0	3	6
31-40	5	2	3	1	11
41-50	9	4	5	5	23
51-60	4	4	8	4	20
>60	0	1	0	1	2
Total	**18**	**14**	**16**	**14**	**62**

Among the 62 samples, maximum number of samples belonged to age group of 41- 50 years of age followed by 51- 60 years of age.

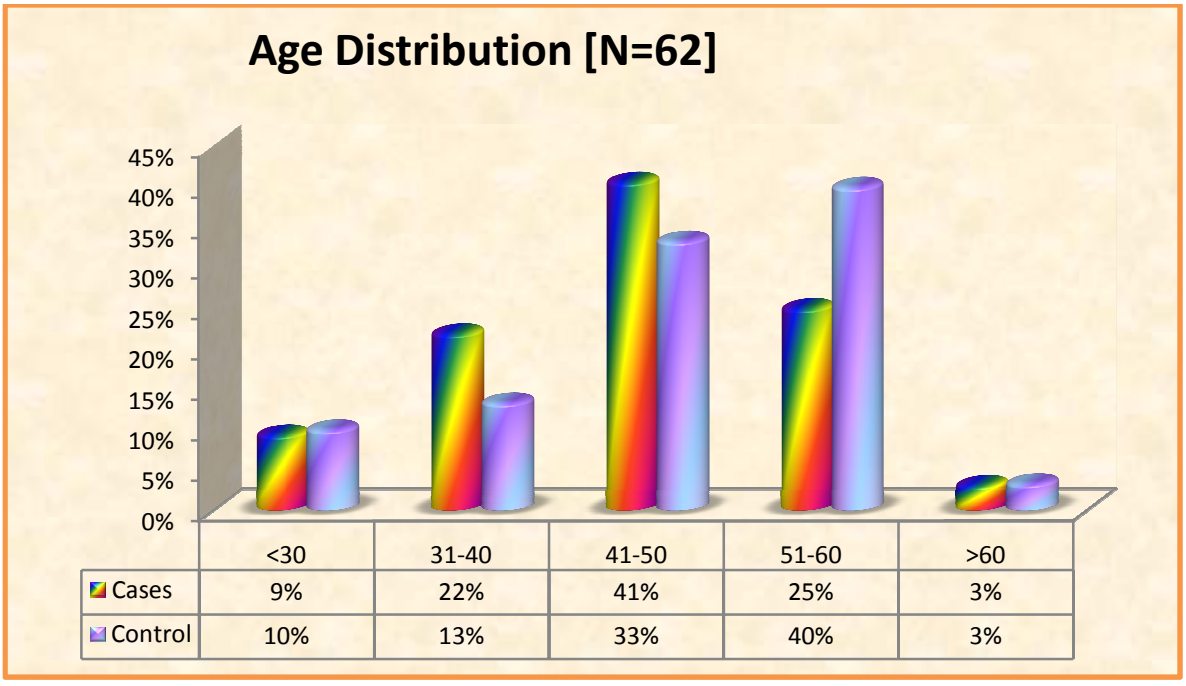
AGE WISE DISTRIBUTION OF CASES AND CONTROLS.

CHART 2a



	Male	Female	Male	Female
	Experimental [n=32]		Control[n=30]	
<30	0%	9%	0%	10%
31-40	16%	6%	10%	3%
41-50	28%	13%	17%	17%
51-60	13%	13%	27%	13%
>60	0%	3%	0%	3%

CHART 2b



	<30	31-40	41-50	51-60	>60
Cases	9%	22%	41%	25%	3%
Control	10%	13%	33%	40%	3%

Among the 62 samples, maximum number of samples belonged to age group of 41- 50 years of age followed by 51- 60 years of age.

TABLE- 5

MEAN AGE WITH GENDER DISTRIBUTION

GROUP	Gender	Mean	SD	Lower	Upper	Minimum	Maximum
Cases	Male	46	8	42	49	33	59
	Female	44	11	38	51	27	63
	Total	**45**	**9**	**42**	**48**	**27**	**63**
Controls	Male	49	9	44	53	34	58
	Female	45	12	38	51	26	61
	Total	**47**	**10**	**43**	**51**	**26**	**61**

In the present study of 62 samples (32 cases and 30 controls), mean age of distribution is 45 years. Among the cases maximum number of patients belonged to 41 to 50 years of age (41%) and maximum number of controls belonged to 51 – 60 years(40%)(chart 1).

TABLE - 6

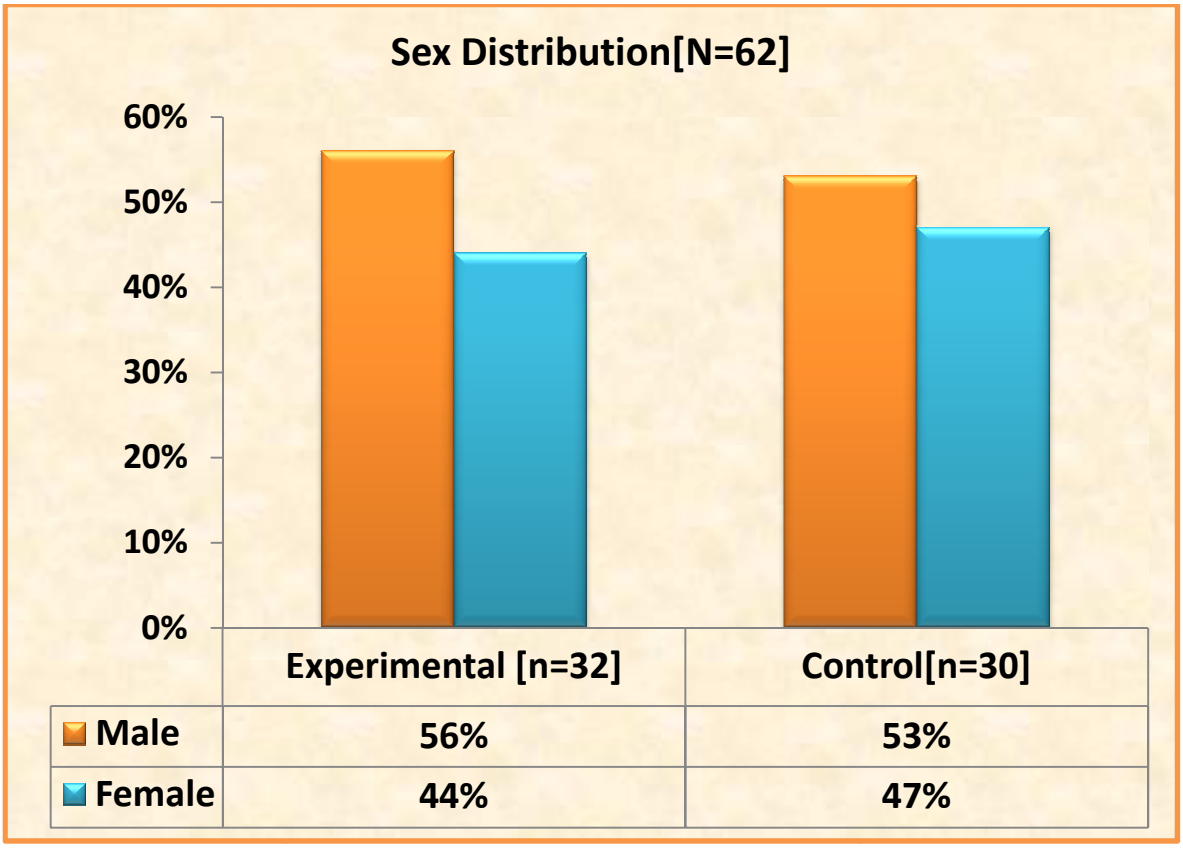
GENDER WISE DISTRIBUTION OF SAMPLES

Gender	Cases	Controls
Male	18	16
Female	14	14
Total	**32**	**30**

In the present study among cases male to female ratio was1.2:1 . Among controls male to female ratio was 1.1:1.

CHART - 3

GENDER WISE DISTRIBUTION OF PSORIASIS



In the present study among cases male to female ratio was1.2:1 with male predominance . Among controls male to female ratio was 1.1:1.

TABLE - 7

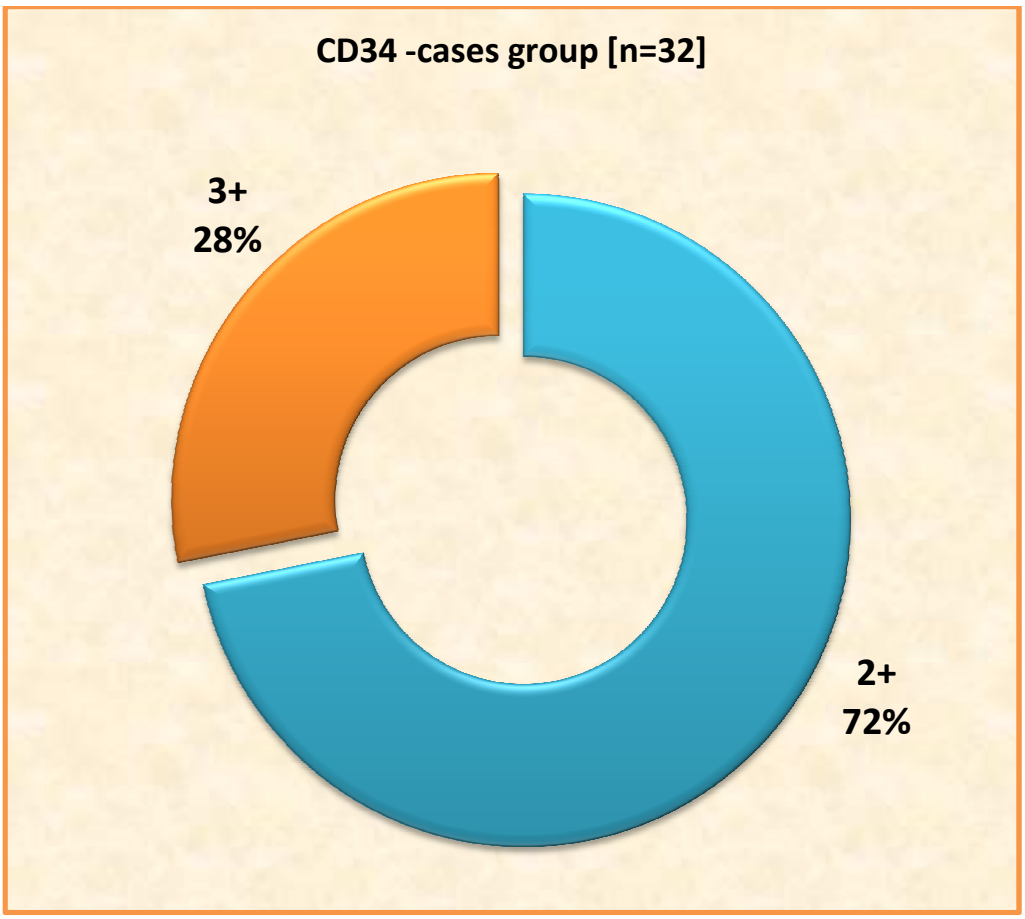
MICROVESSEL DENSITY - EVALUATION OF CD 34 STAINING

CD34	Cases	Control	Total	(%)
Neg	0	23	23	37%
1+(mild)	0	7	7	11%
2+(moderate)	23	0	23	37%
3+(severe)	9	0	9	15%
Total	32	30	62	100%

CD 34 expression was seen in all samples from psoriatic skin lesions with various intensities. CD34 expression was weak in all control skin samples. Among cases 72% of skin samples showed moderate degree of staining.

CHART - 4a

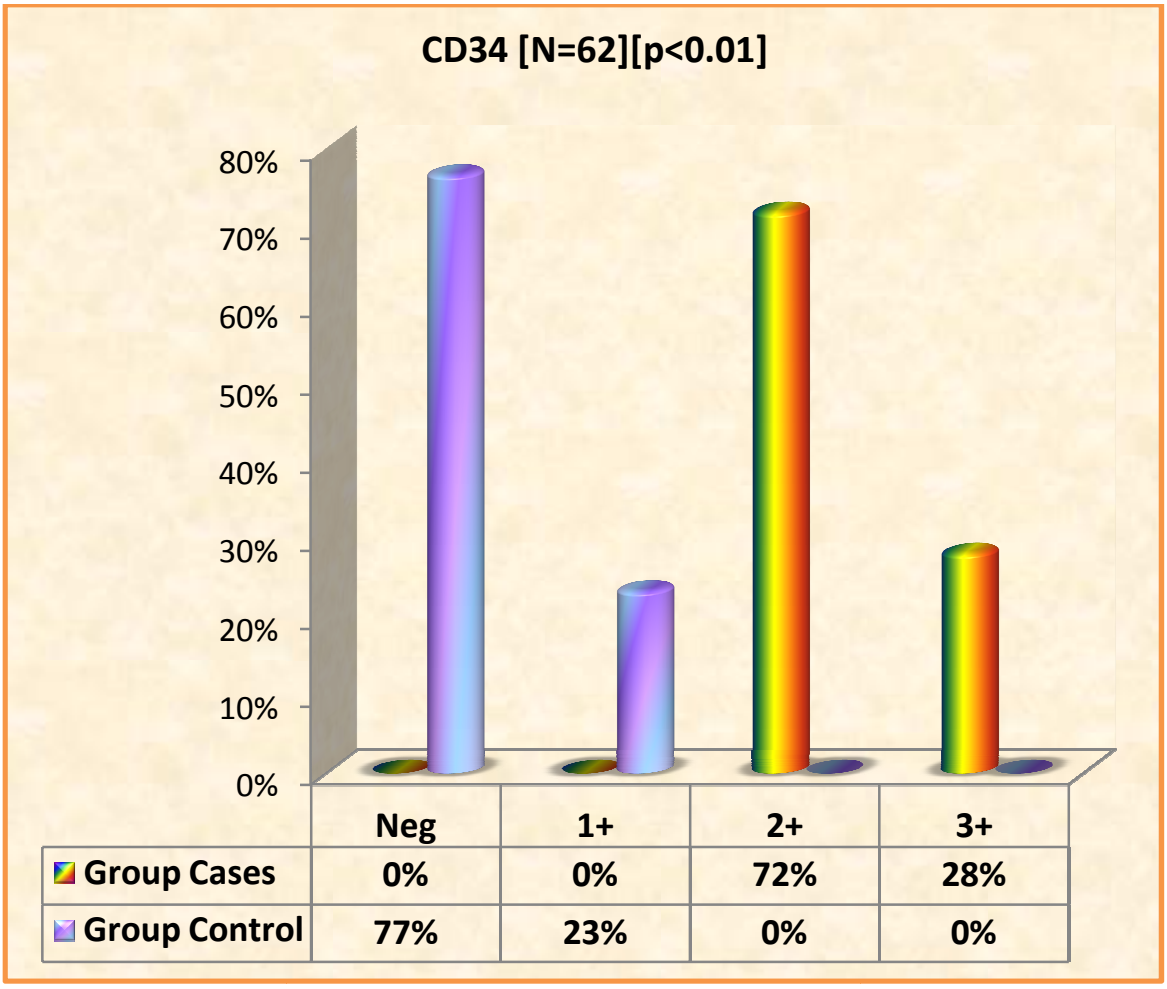
INTENSITY OF CD34 STAINING AMONG CASES



Among cases 72% of skin samples showed moderate degree of staining for CD 34.

CHART - 4b

COMPARISON OF IMMUNOREACTIVITY OF CD 34 BETWEEN CASES AND CONTROL



	Neg	1+	2+	3+
Group Cases	0%	0%	72%	28%
Group Control	77%	23%	0%	0%

CD34 expression was weak in all control skin samples .among controls 23% showed weak positivity. Among cases 72% of skin samples showed moderate degree of staining and 28% with higher intensity of staining.

TABLE - 8

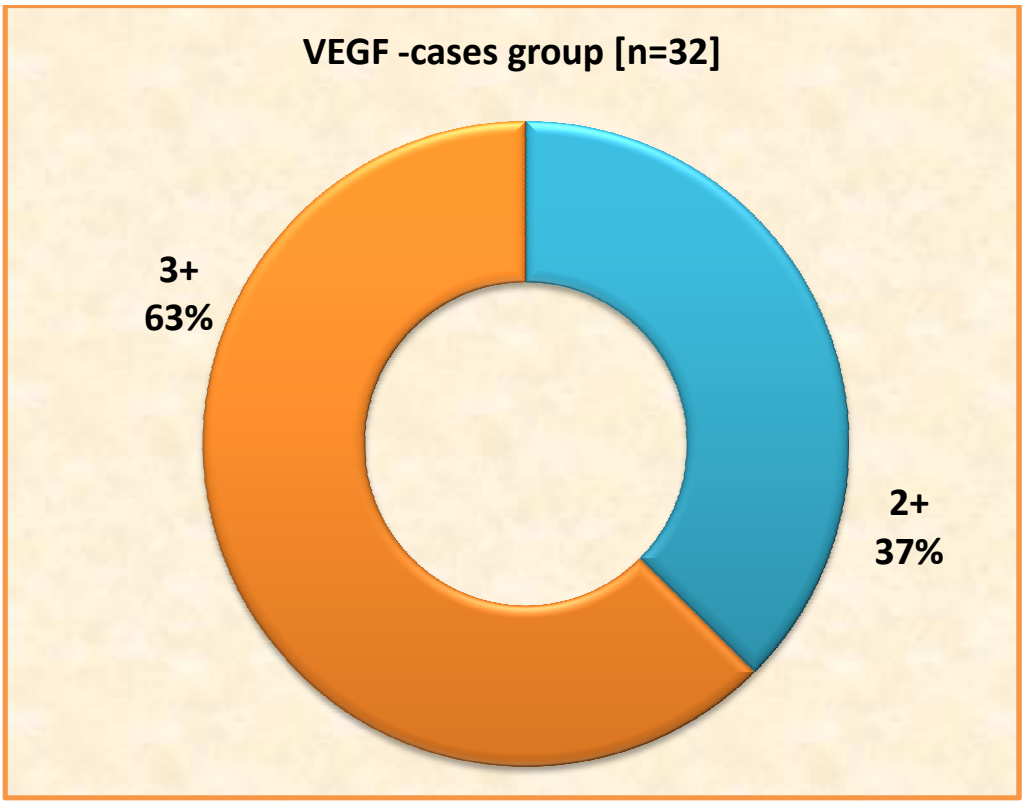
IMMUNOHISTOCHEMICAL STAINING OF VEGF

VEGF	Cases	Control	Total	(%)
Neg	0	23	23	37%
1+	0	7	7	11%
2+	12	0	12	19%
3+	20	0	20	32%
Total	32	30	62	100%

VEGF expression was seen in all cases and weak staining in controls. 63% of cases showed diffuse staining of full thickness of epidermis with only 23% of controls showing weak positivity of epidermis.

CHART 5a

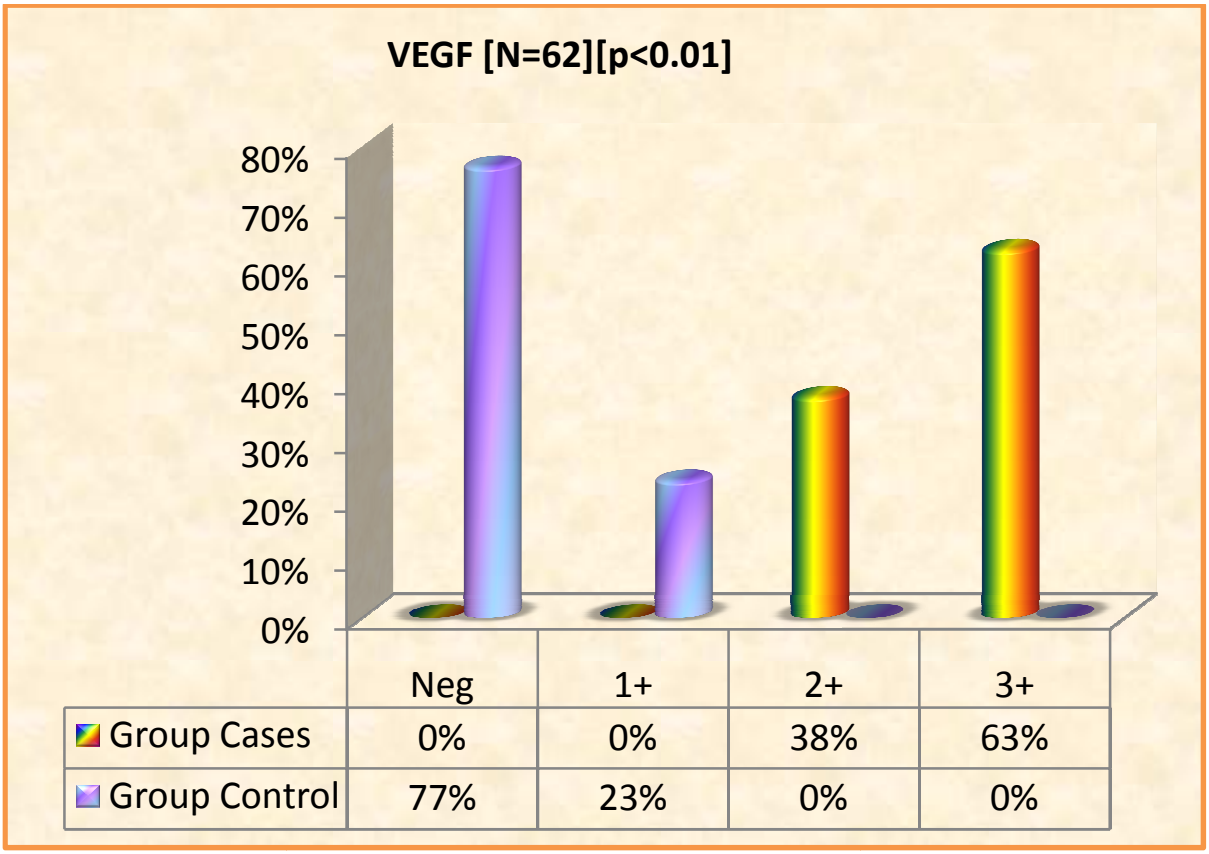
IMMUNOREACTIVITY OF VEGF AMONG CASES



63% of cases showed diffuse staining of VEGF involving full thickness of epidermis

CHART - 5b

COMPARISON OF VEGF EXPRESSION BETWEEN CASES AND CONTROL



	Neg	1+	2+	3+
Group Cases	0%	0%	38%	63%
Group Control	77%	23%	0%	0%

VEGF expression was seen in all cases with a weak staining in controls. 63% of cases showed diffuse staining of full thickness of epidermis with only 23% of controls showing weak positivity in epidermis.

TABLE - 9

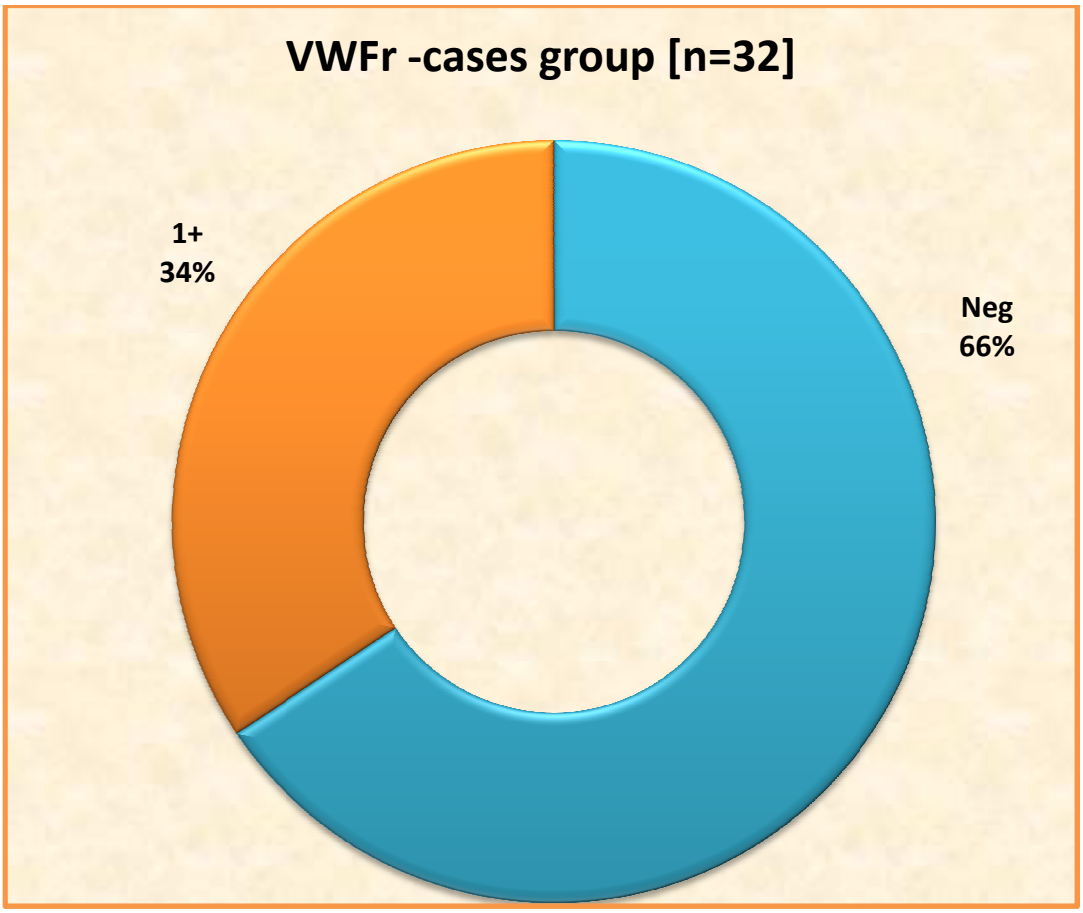
IMMUNOHISTOCHEMICAL EVALUATION OF VONWILLEBRAND FACTOR

VWFr	Cases	Control	Total	(%)
Neg	21	20	41	66%
1+	11	10	21	34%
Total	**32**	**30**	**62**	**100%**

Vonwillebrand factor showed similar weak epidermal expression in both cases and control group. Cases with negative staining were 66% and 67% of controls showed negative expression.

CHART - 6a

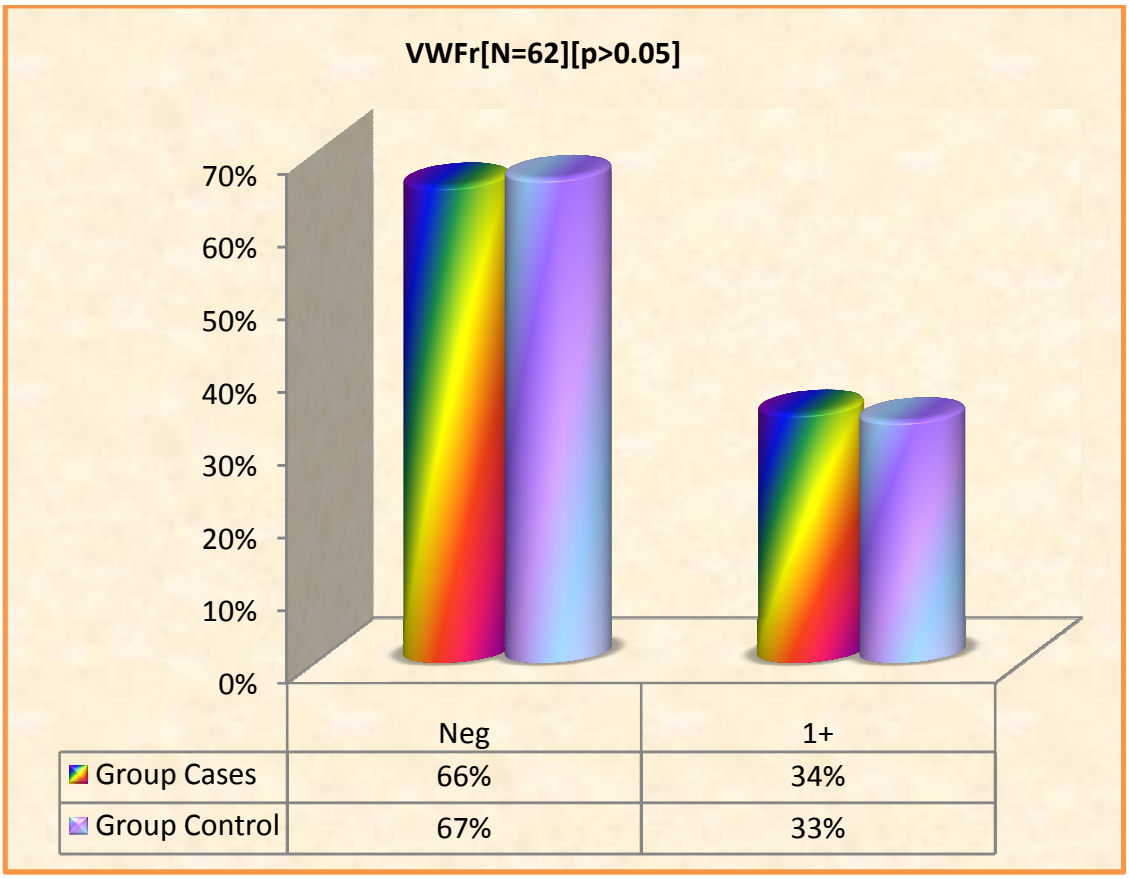
INTENSITY OF VWFr STAINING AMONG CASES



Cases with negative staining for vonwillebrand factor were 66%

CHART - 6b

COMPARISON OF VWFr EXPRESSION BETWEEN CASES AND CONTROL



Vonwillebrand factor showed similar type of weak epidermal expression in both case group and control group. Cases with negative staining were 66% and 67% of controls showed negative expression

TABLE - 10

MEAN OF ANGIOGENIC FACTOR EXPRESSION

Factors	Group	Mean	SD	Lower	Upper	Minimum	Maximum	P value
PASI	Case	16.2	1.9	15.5	16.9	13.8	19.6	
CD34	Case	2.3	0.5	2.1	2.5	2.0	3.0	< 0.05
	Control	0.2	0.4	0.1	0.4	0.0	1.0	
	Total	**1.3**	**1.1**	**1.0**	**1.6**	**0.0**	**3.0**	
VEGF	Case	2.6	0.5	2.5	2.8	2.0	3.0	<0.05
	Control	0.2	0.4	0.1	0.4	0.0	1.0	
	Total	**1.5**	**1.3**	**1.1**	**1.8**	**0.0**	**3.0**	
VWFr	Case	0.34	0.48	0.2	0.5	0.0	1.0	> 0.05
	Control	0.33	0.48	0.2	0.5	0.0	1.0	
	Total	**0.3**	**0.5**	**0.2**	**0.5**	**0.0**	**1.0**	

I. Mean expression of CD 34 among cases was 2.3 +/-0.5 SD and among controls 0.2+/- 0.4SD.

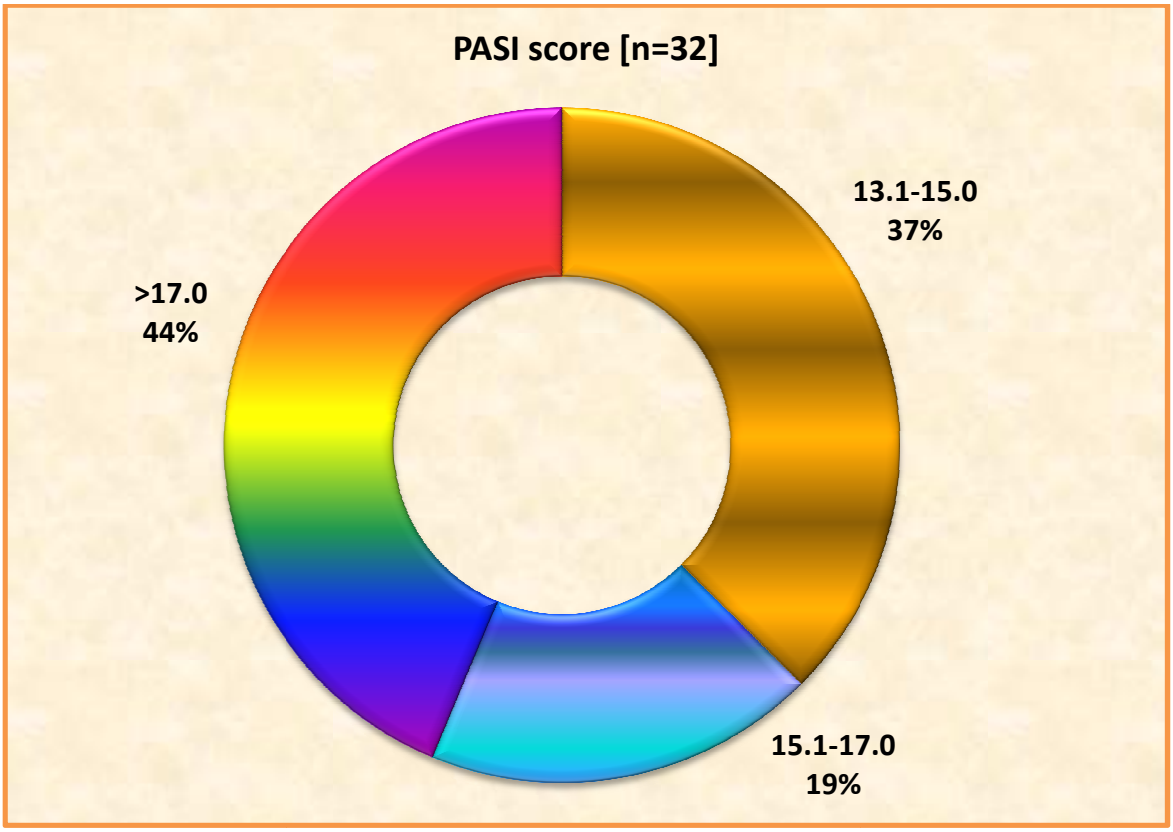
II. Mean expression of VEGF among cases was 2.6 +/0.5- SD and among controls 0.2+/- 0.4SD.

III. Mean expression of VWFr among cases was 0.34 +/-0.48 SD and among controls 0.33+/- 0.48SD.

CHART - 7

PASI SCORE – IN CASES

PSORIASIS AREA SEVERITY INDEX



Most of the cases belonged to the PASI score ranging from 13.1 to 15.

TABLE – 11

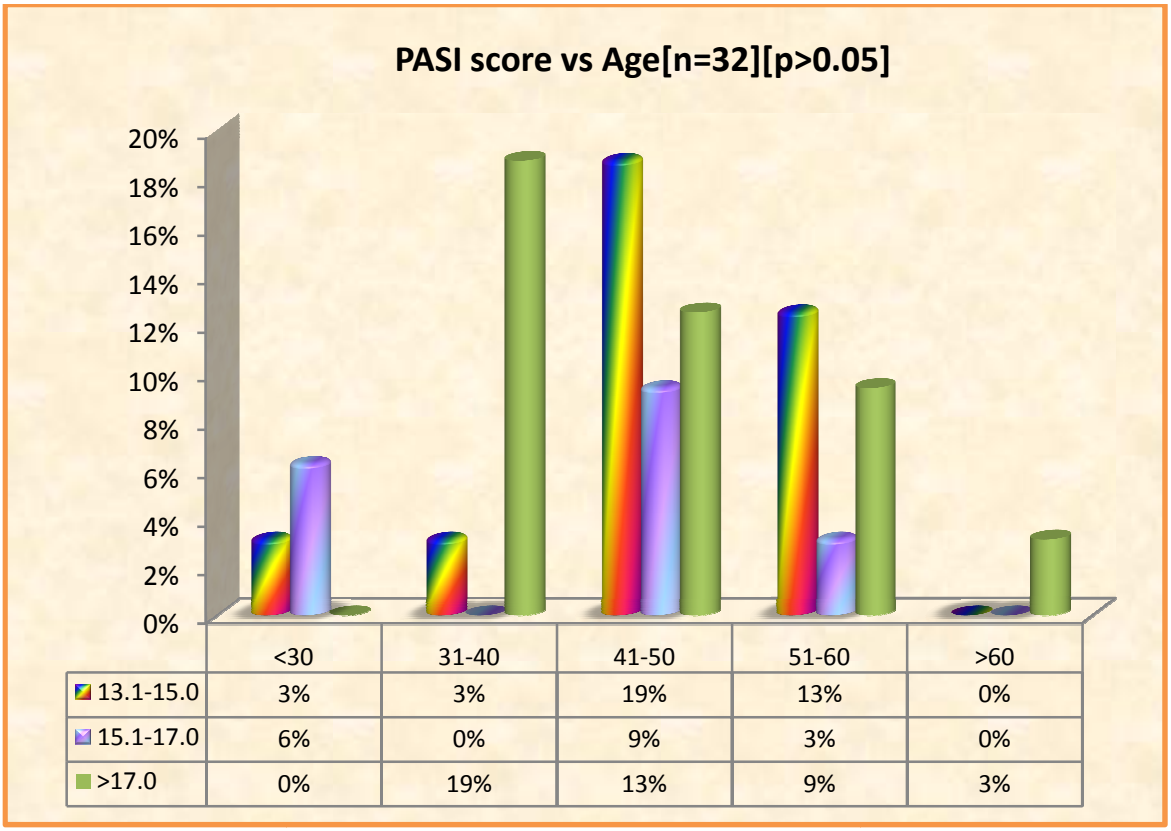
AGE Vs PASI SCORE

Age	PASI Score			
	13.1-15.0	**15.1-17.0**	**>17**	**Total**
<30	1	2	0	3
31-40	1	0	6	7
41-50	6	3	4	13
51-60	4	1	3	8
>60	0	0	1	1
Total	12	6	14	32

The mean PASI score in the present study was 16.2+/-1.9

CHART 8

AGE Vs PASI SCORE



	<30	31-40	41-50	51-60	>60
13.1-15.0	3%	3%	19%	13%	0%
15.1-17.0	6%	0%	9%	3%	0%
>17.0	0%	19%	13%	9%	3%

The mean PASI score in the present study was 16.2+/-1.9

TABLE - 12

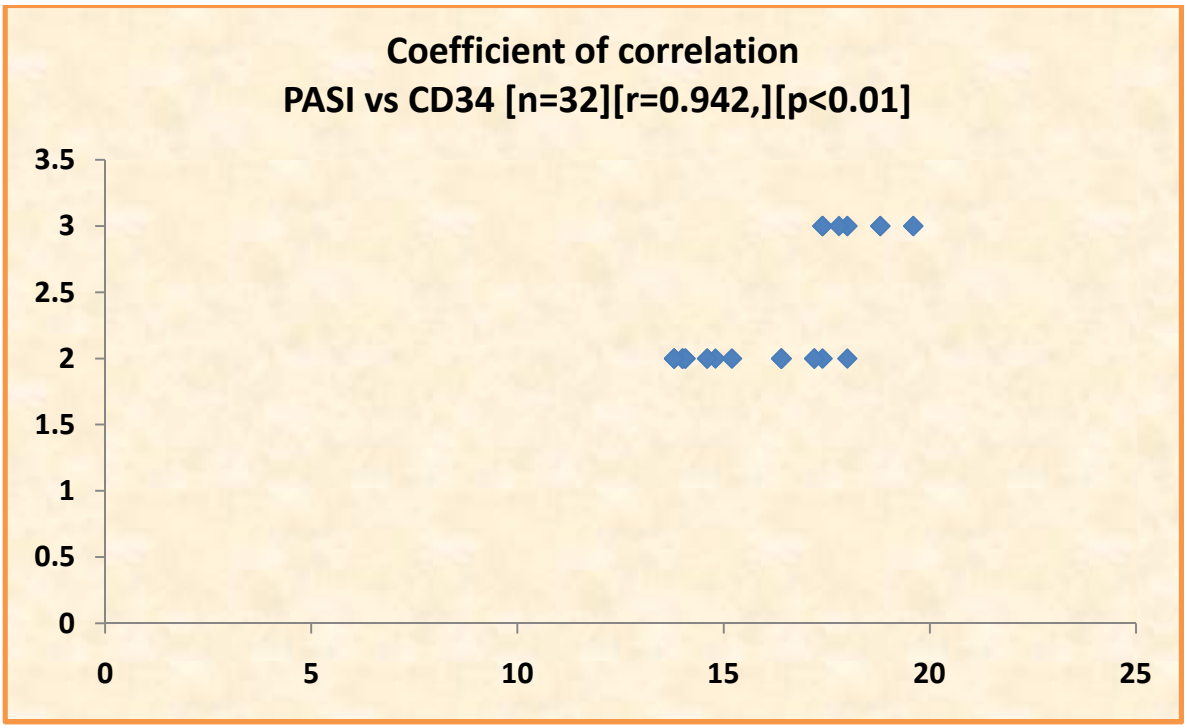
CORRELATION BETWEEN PASI SCORE AND ANGIOGENIC FACTORS

		PASI Score	CD 34	VEGF	VWFr.
PASI Score	Pearson Correlation	1	.942**	.944**	0.024
	Sig. (2-tailed)		0.000	0.000	0.855
	N	62	62	62	62
CD 34	Pearson Correlation	.942**	1	.879**	0.058
	Sig. (2-tailed)	0.000		0.000	0.653
	N	62	62	62	62
VEGF	Pearson Correlation	.944**	.879**	1	-0.022
	Sig. (2-tailed)	0.000	0.000		0.866
	N	62	62	62	62
VWFr.	Pearson Correlation	0.024	0.058	-0.022	1
	Sig. (2-tailed)	0.855	0.653	0.866	
	N	62	62	62	62

There was significant correlation between expression of angiogenic factors and psoriatic area and severity index(PASI Score)

CHART - 9

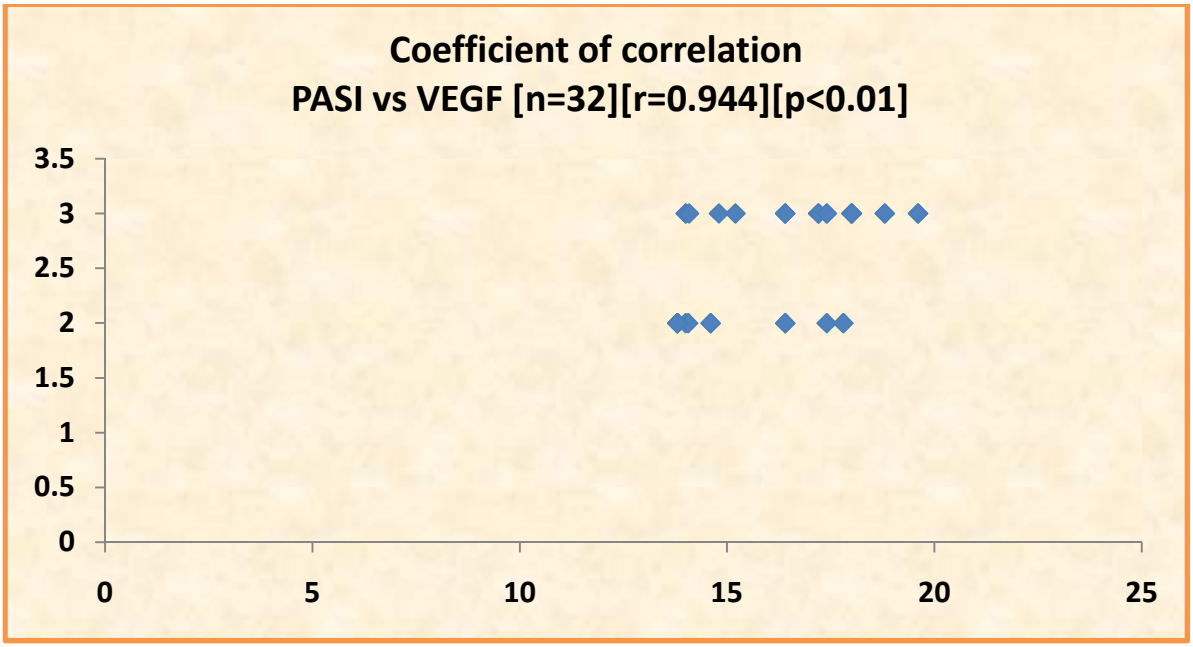
CORRELATION BETWEEN PASI AND CD34



There is significant correlation between expression of CD34 with psoriatic area snd severity index score and it was statistically significant.

CHART - 10

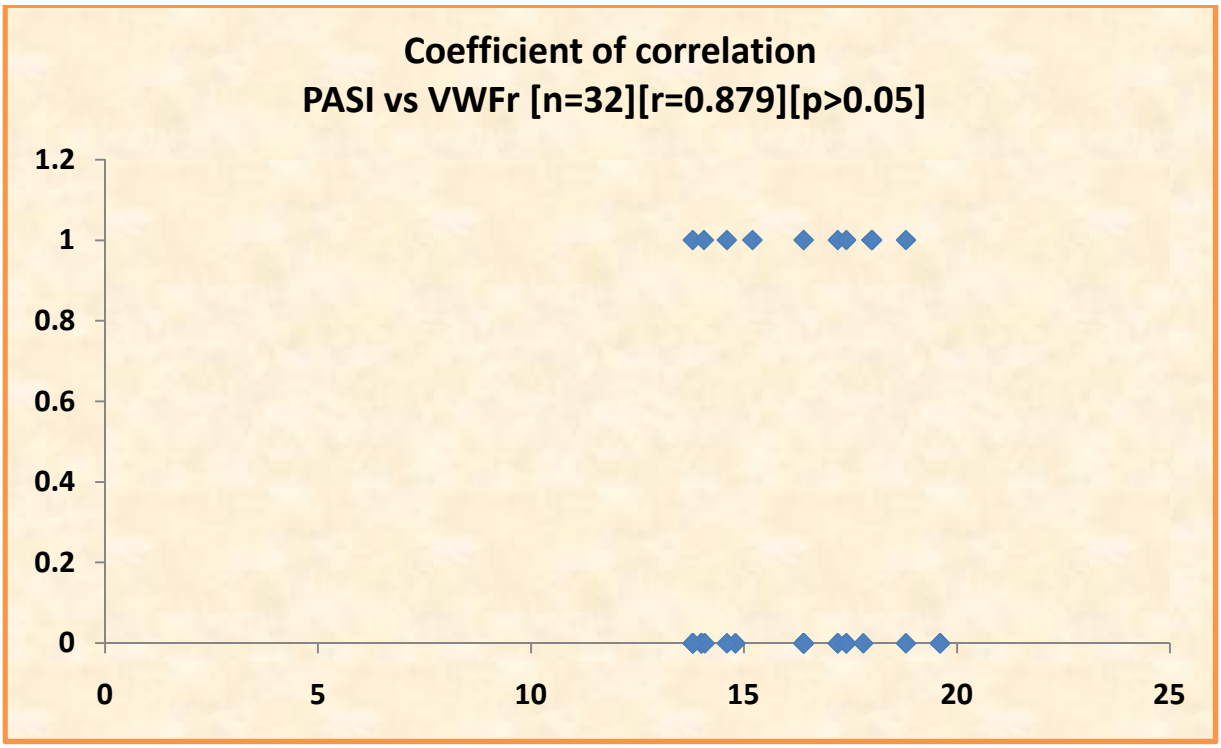
CORRELATION BETWEEN PASI AND VEGF



There is a significant correlation between expression of VEGF with psoriatic area snd severity index score and it was statistically significant.

CHART - 11

CORRELATION BETWEEN PASI AND VWFr



There is no statistically significant correlation between expression vWFr with PASI score.

STATISTICAL ANALYSIS

Data obtained was coded and entered into Microsoft excel spread sheet. The data are reported as the mean +/- SD or the median , depending on their distribution. Qualitative variables were presented as number and percent. The differences in quantitative variables between groups were assessed by means of the unpaired t test. Comparison between groups were made by the Non parameteric Mann – whitney test. A chisquare test was used to assess differences in categoric variables between groups. Pearson's coefficients of correlation was used to assess the relationship between the variables.

A p value of <0.05 using a two tailed test was taken as being significance for all statistical tests.

All data were analysed with a statistical software package (SPSS, version 16.0 for windows)

Colour Plates

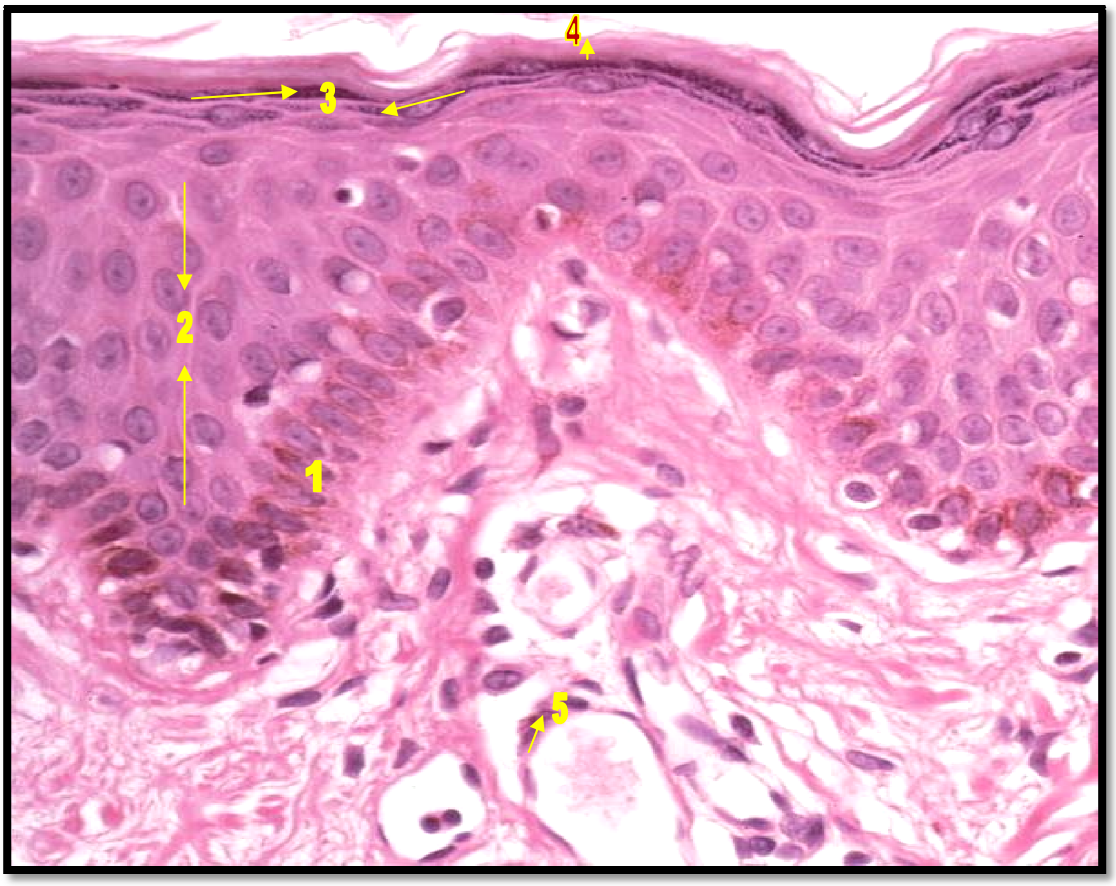


FIG 1 NORMAL HISTOLOGY OF SKIN

1 - stratum basale

2 - stratum spinosum

3 - stratum granulosum

4 - stratum corneum

5 - dermal capillaries

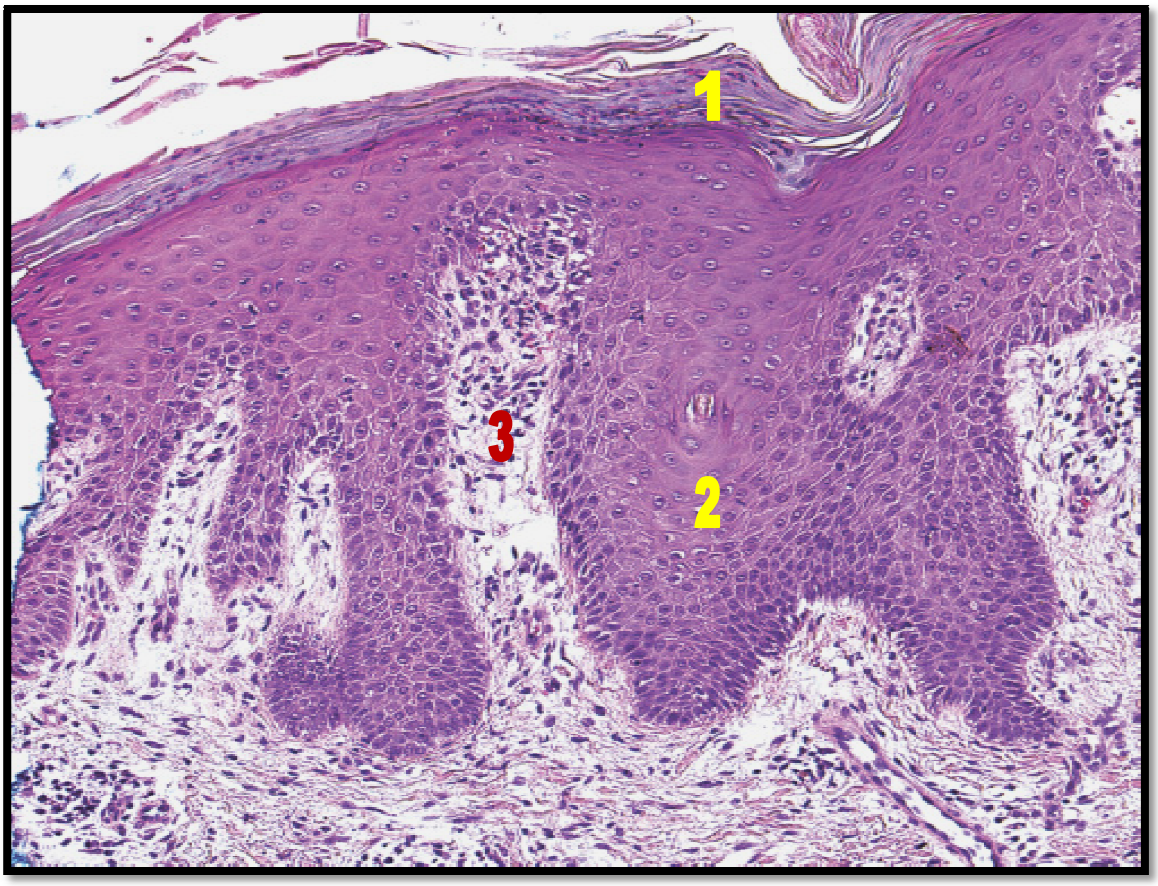


FIG 2 HISTOPATHOLOGY OF PSORIASIS

1 - hyperkeratosis with munro s abscess

2 - psoriasiform hyperplasia

3 - dilated and tortuous dermal capillaries

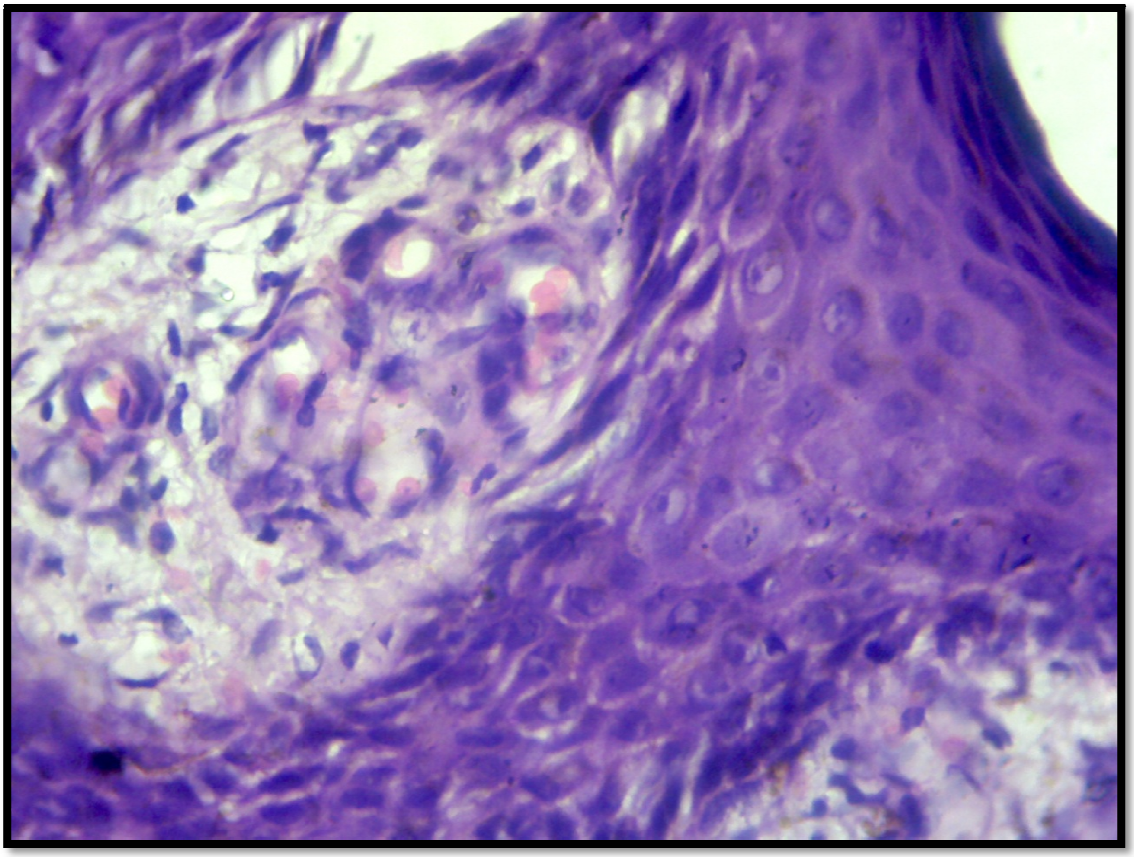


FIGURE 3 – DILATED DERMAL CAPILLARIES IN PSORIASIS

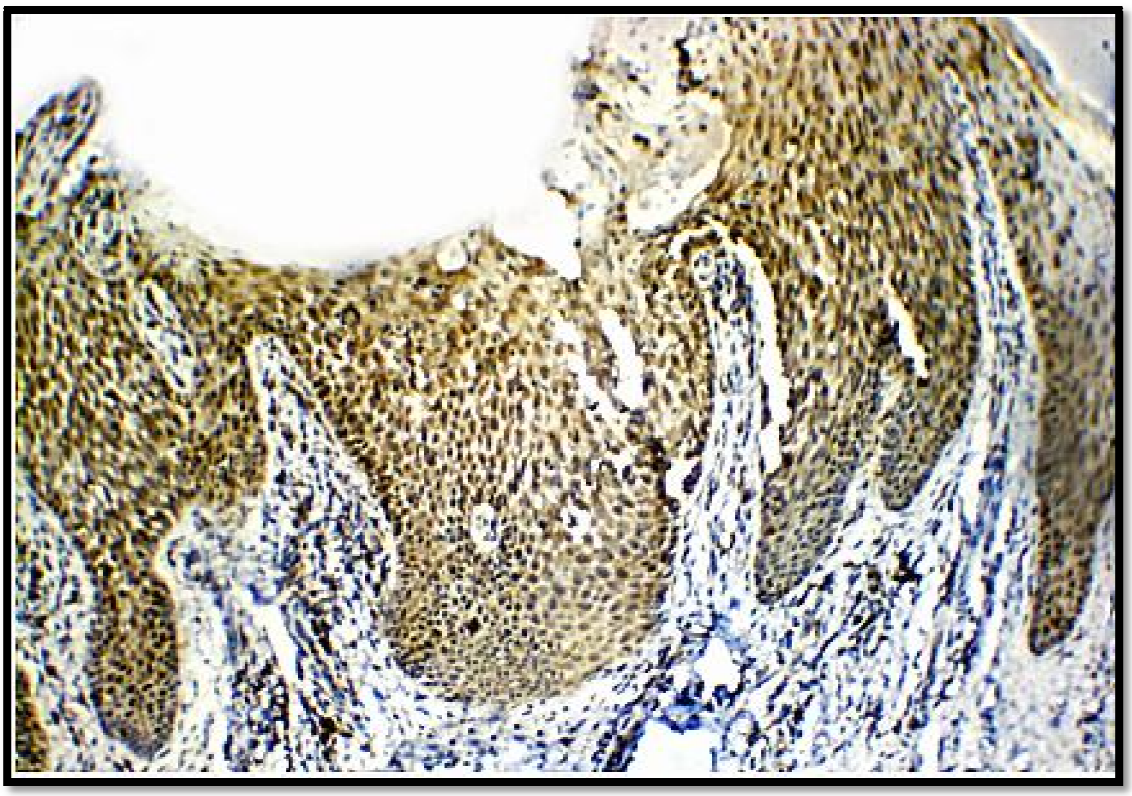


FIG 4: IHC SHOWS LOW POWER - VEGF MODERATE EPIDERMAL POSITIVITY (2+) IN CASES

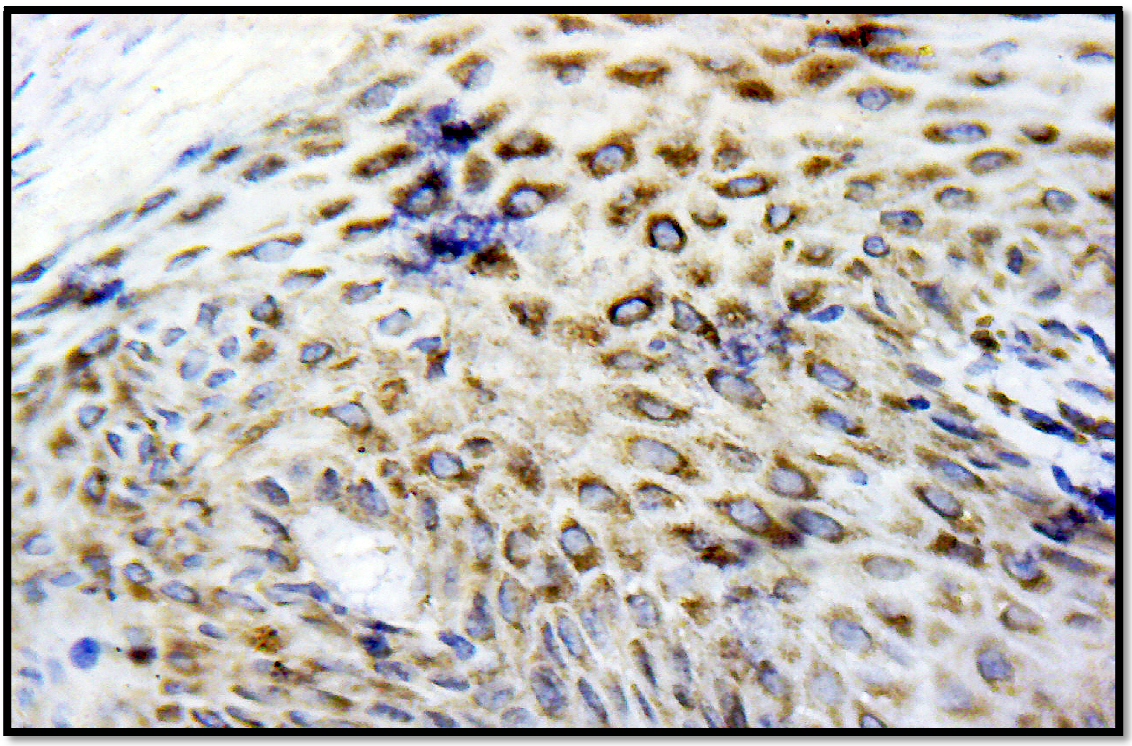


FIG 5 : IHC HIGH POWER SHOWS MODERATE DEGREE OF EPIDERMAL STAINING (2+) IN CASES

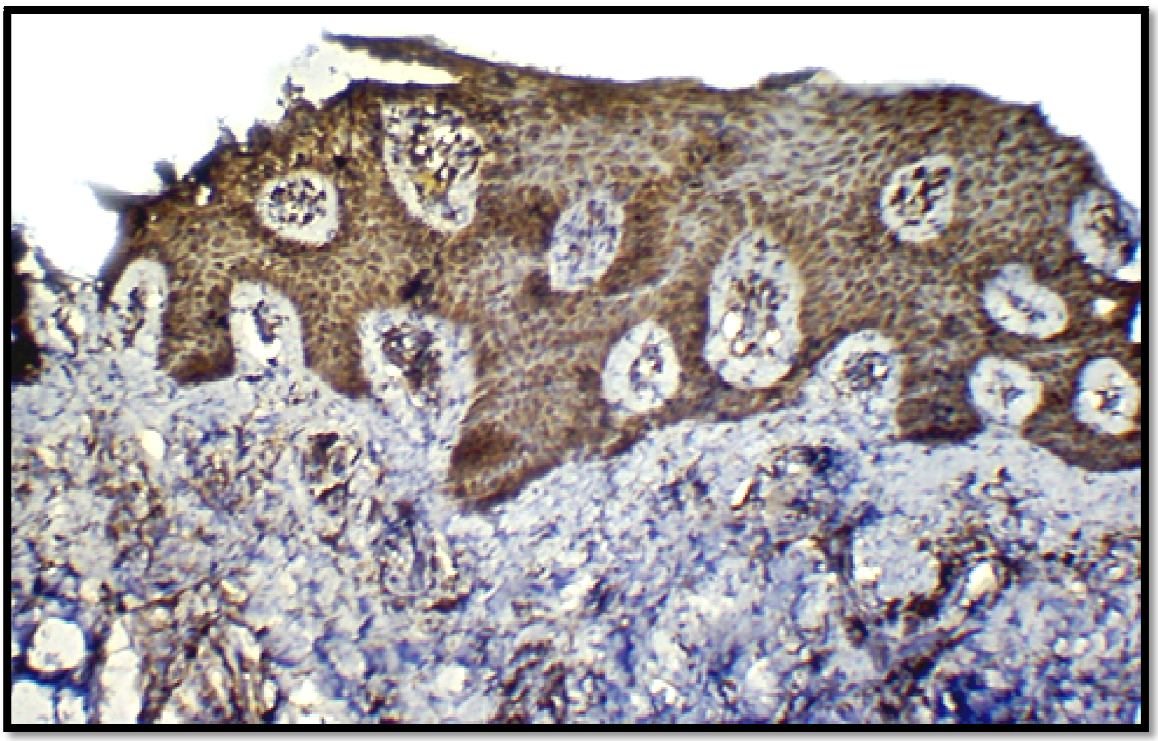


FIG 6 : IHC LOW POWER SHOWS DIFFUSE EPIDERMAL STAINING OF VEGF (3+ POSITIVITY) IN CASES

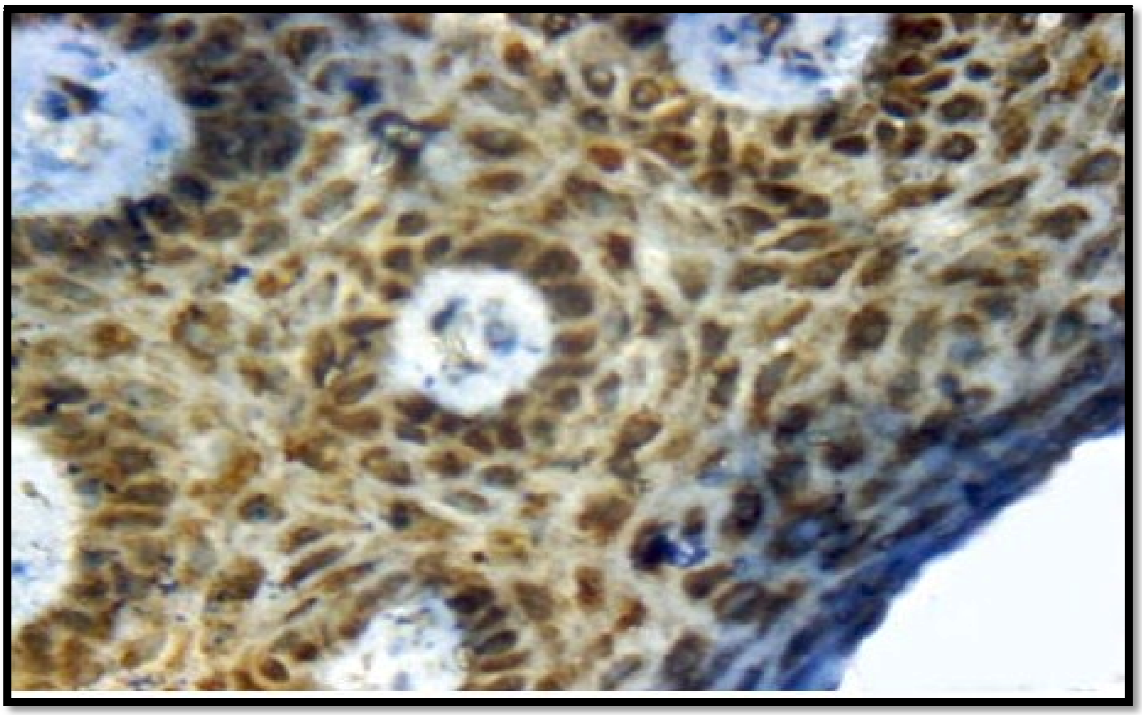


FIG 7 : IHC HIGH POWER SHOWS CYTOPLASMIC AND MEMBRANOUS POSITIVITY - HIGHER DEGREE (3+) IN CASES

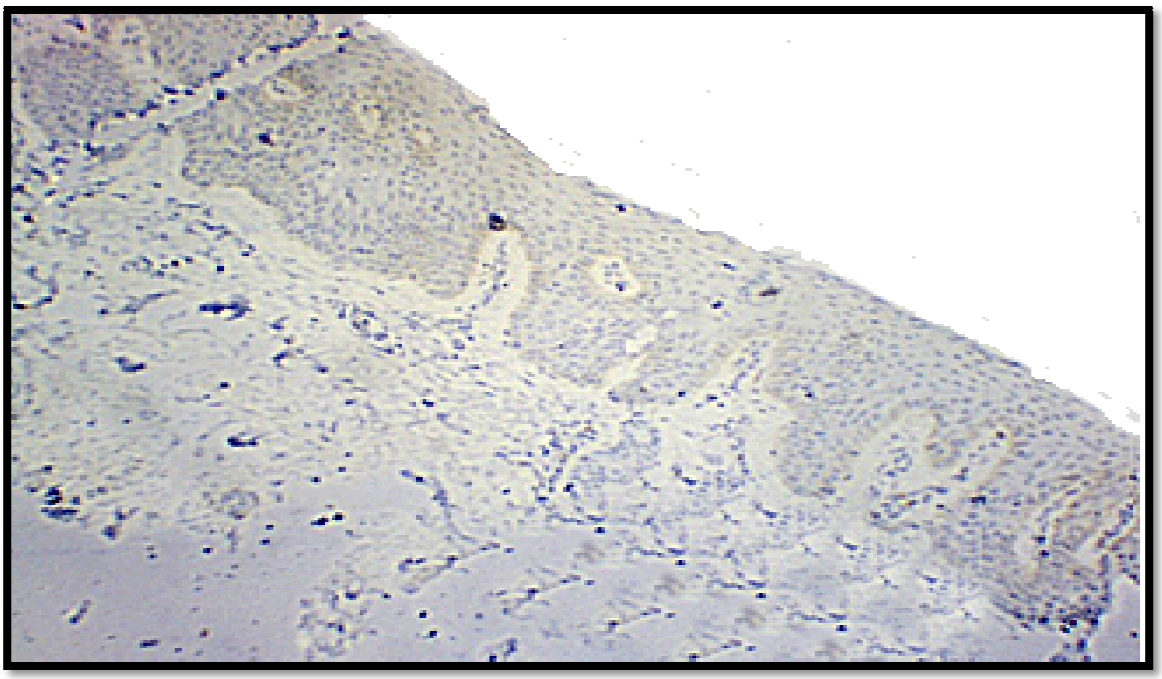


FIG 8: IHC LOW POWER - SHOWS VEGF WEAK EPIDERMAL STAINING (1+) IN CONTROLS

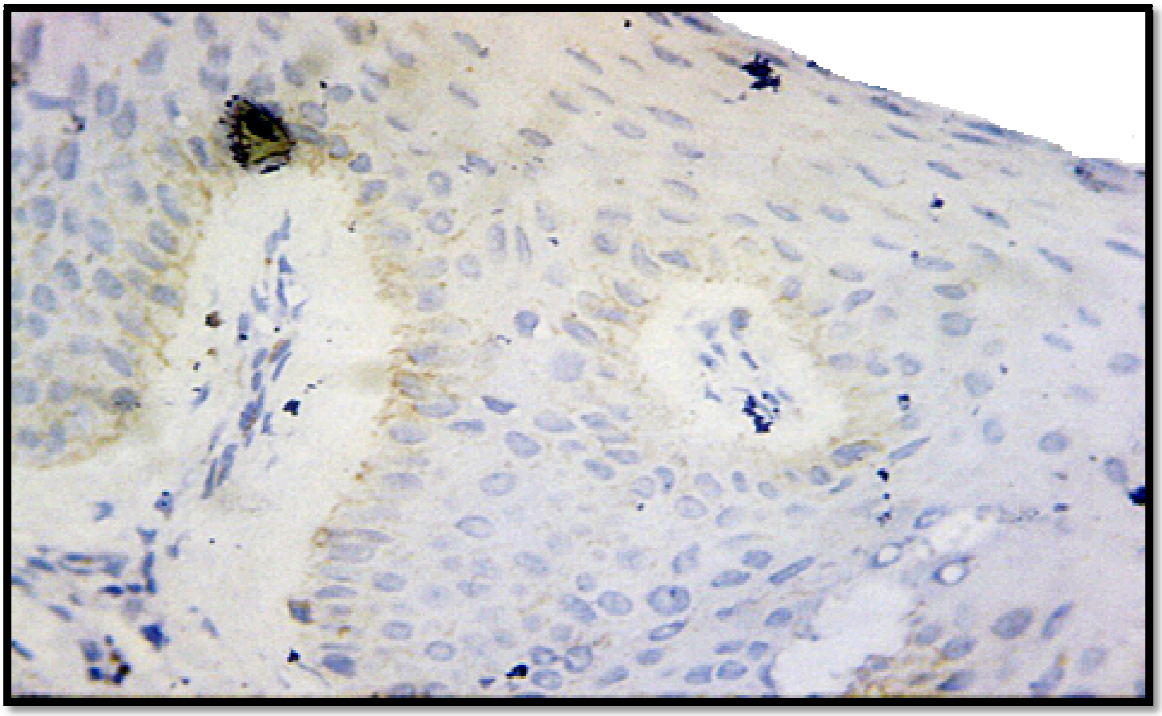


FIG 9 IHC HIGH POWER SHOWS WEAK EPIDERMAL POSITIVITY OF VEGF (1+) IN CONTROLS

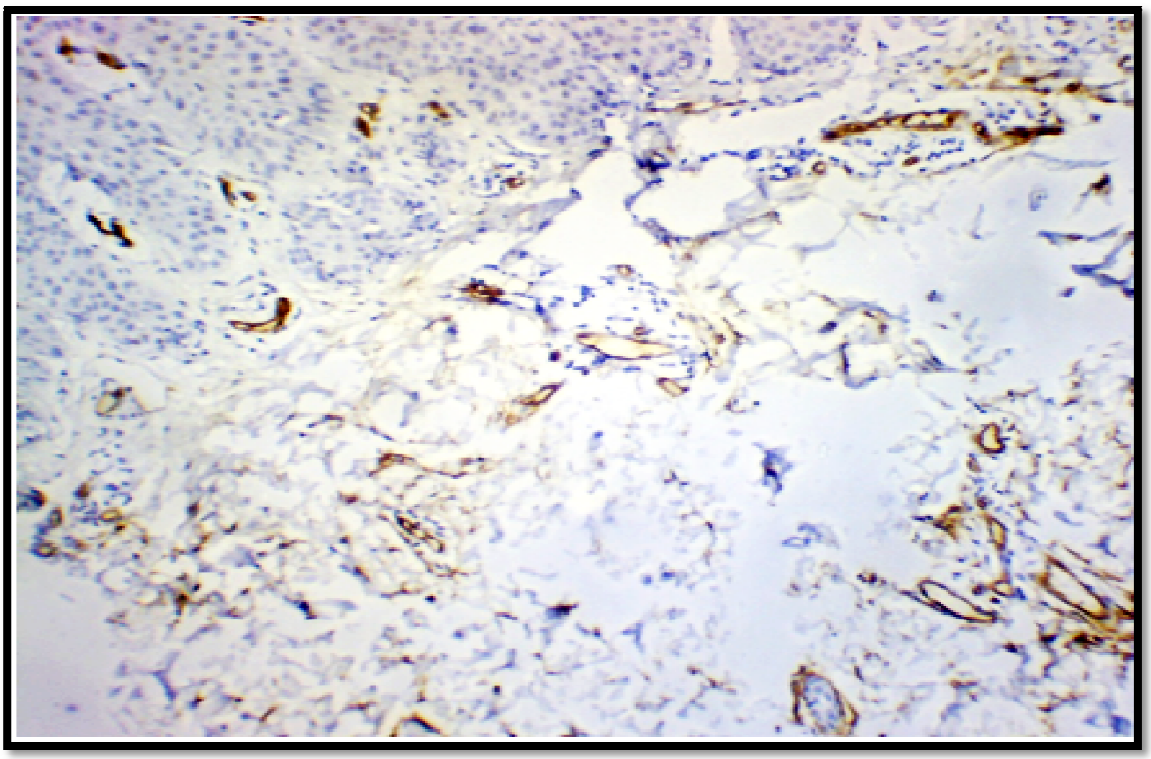


FIG 10: IHC LOW POWER VIEW SHOWS CD34 POSITIVITY IN CASES

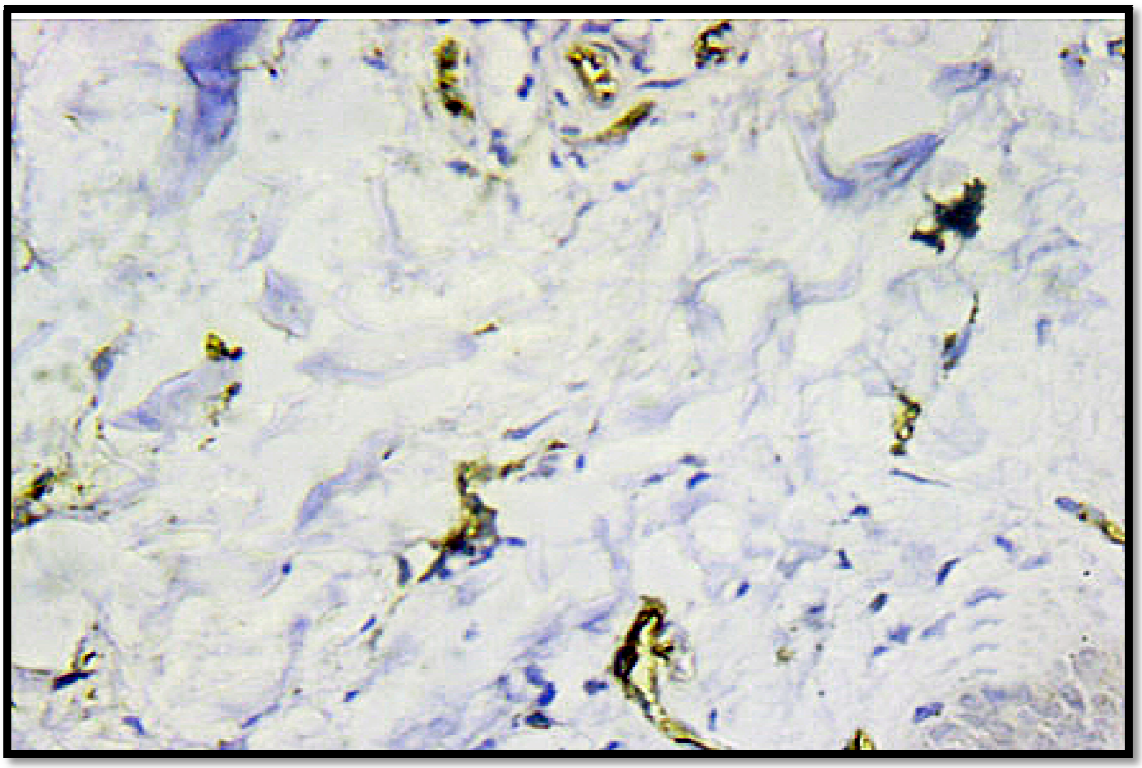


FIG 11: IHC HIGH POWER VIEW SHOWS CD 34 MILD POSITIVITY (1+) IN CASES

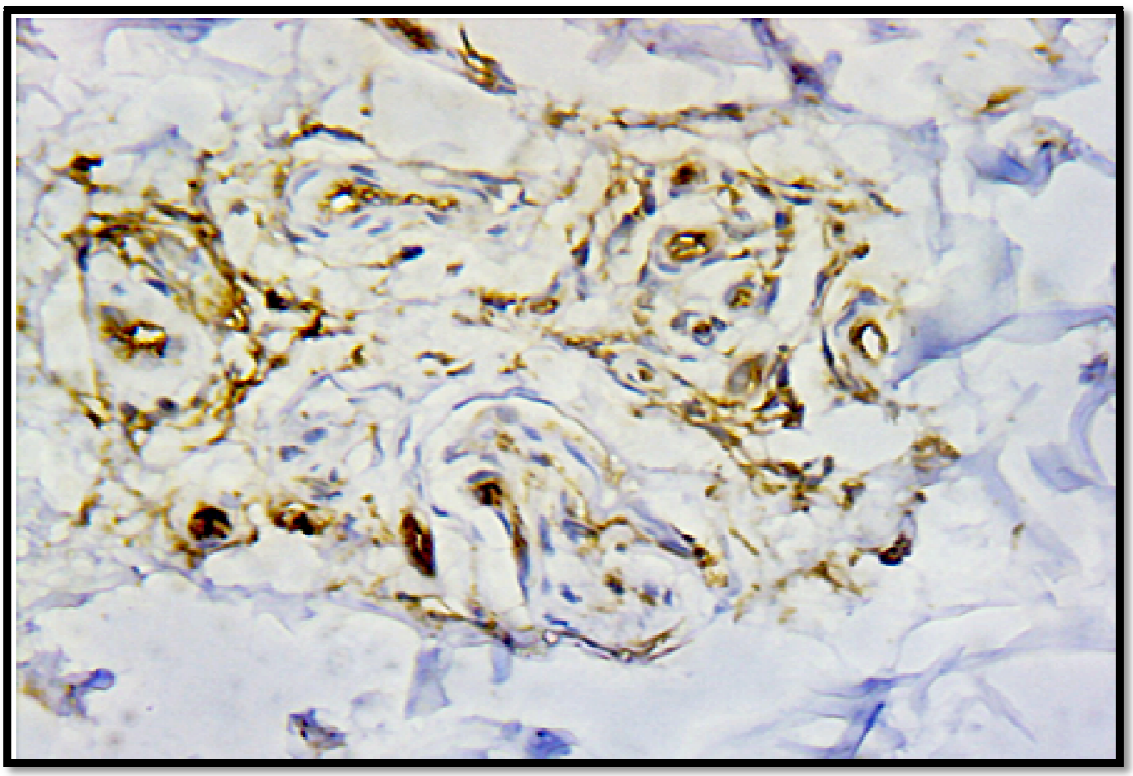


FIG 12: IHC HIGH POWER VIEW SHOWS MODERATE DEGREE OF POSITIVITY (2+) IN CASES

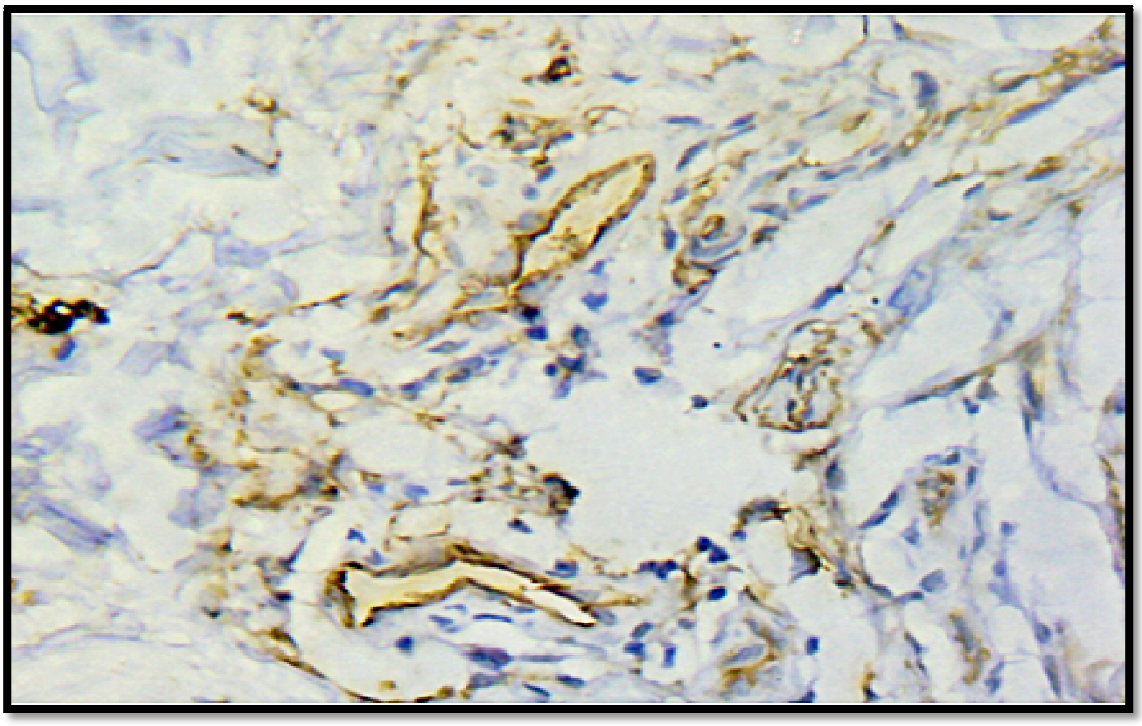


FIG :13 IHC HIGH POWER VIEW SHOWS HIGHER DEGREE OF POSITIVITY (3+) IN CASES

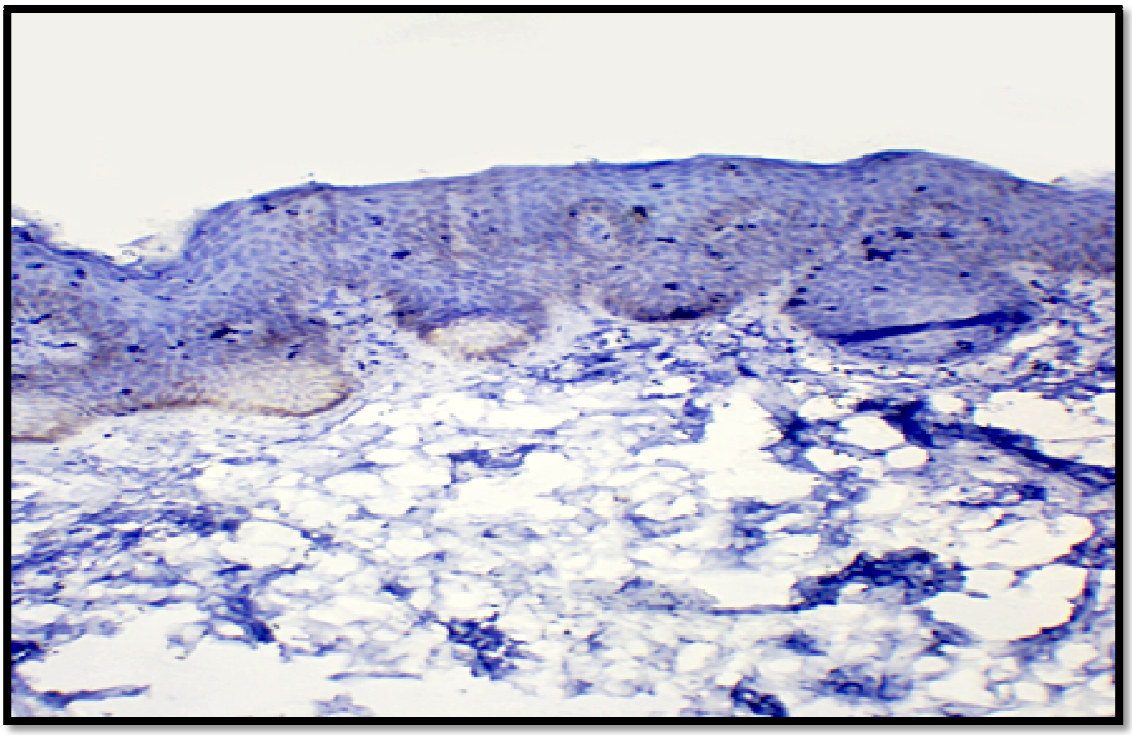


FIG 14: LOW POWER SHOWS WEAK EPIDERMAL STAINING OF VON WILLE BRAND FACTOR IN CASES

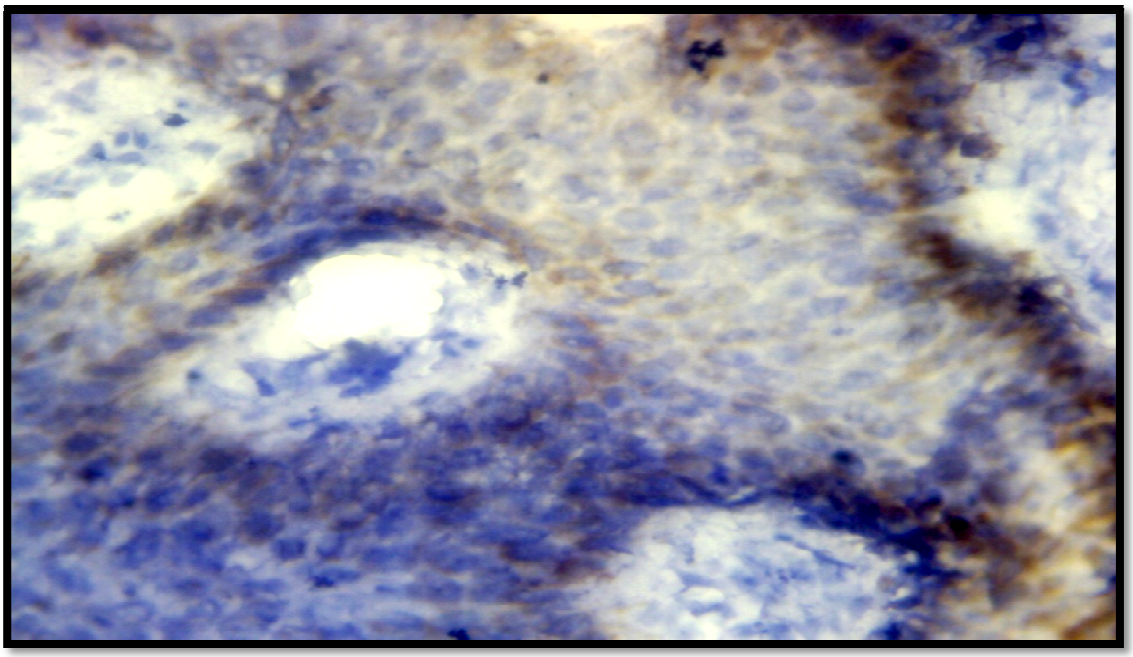


FIG 15: HIGH POWER SHOWS WEAK EPIDERMAL STAINING OF VON WILLE BRAND FACTOR IN CASES

Discussion

DISCUSSION

Psoriasis is an autoimmune chronic inflammatory dermatological disease. It affects people of all age groups. In case of genetic transmission it can have onset in early adulthood. Clinically psoriasis vulgaris which is a chronic plaque type of skin lesion presenting as raised, reddish scaly lesion.. Histopathologically , psoriasis is characterised by hyperkeratosis, parakeratosis, epidermal hyperplasia spongiform pustules of kogoj, suprapapillary thinning of granular layer, inflammatory infiltrate in dermis and capillary proliferation in dermis.

The pathogenesis is yet to be elucidated. Various factors seem to play a pathogenetic role in psoriasis. They are immune mediated aberrant regulation of T lymphocytes, interaction between rapidly proliferating epidermal cells and an array of cytokines, angiogenesis, neovascularisation and vascular remodelling. Several studies show that rapidly proliferating keratinocytes seem to secrete certain cytokines and angiogenic factors such as VEGF. Angiogenesis refers to development of new blood vessels from the preexisting vascular channels. In Psoriasis there is marked increase in endothelial microvasculature thus supporting that psoriasis is angiogenesis dependent. Microvascular expansion in dermis with abnormal dilatation and orientation of capillaries in skin

biopsy in psoriasis patients signifies the importance that psoriasis is angiogenesis dependent. Psoriasis was studied in the light of its angiogenic nature .

The keratinocytes in psoriasis skin lesion are considered to be a source of proangiogenic cytokines like vascular endothelial growth factor, tumor necrosis factor alpha, endothelial cell stimulating factor and platelet derived growth factor. There are newer angiogenic factors namely vWFr- von willebrand factor, NGF – nerve growth factor[9]. These cytokines stimulate angiogenesis in psoriatic skin lesions.

The present study encompasses the immunohistochemical expression of angiogenic factors and angiogenic markers in cases and controls. The present evaluates the neovasularisation and vascular changes in psoriatic skin lesions.

In the present study the mean age of occurence for the cases was 45 years and mean age of for the controls was 47 years. In a similar study done by Siaw – Cheok[9] et al , the mean age of occurrence for the cases was 47.94 years and mean age for the controls was 42.33 years. In another study done by Moorchung et al[194] the mean age was 38.9 years for cases. .

In the present study there were 18 males and 14 females among cases and among controls there were 16 males and 14 females. In a study done by Moorchung et al[194] there were 48 males and 40 females among cases.

In the present study the average PASI score for cases was 16.2+/-1.9. In a similar study done by Siaw- Cheok et al[9] the average PASI score was 7.247+/-4.780.

In the our study VEGF expression was observed in all cases with various intensities. VEGF expression was weak in most of the controls. VEGF expression was significantly higher in cases compared to controls with a statistical significant difference (p=<0.05) . Similar results were observed in the following studies.

According to a study conducted by Siaw – Cheok[9] et al intensity of VEGF expression was higher in cases compared to controls(p=0.016). Simonetti et al[7] observed that there was diffuse VEGF(13.15+/- 6.6) immunohistochemical expression in epidermis of psoriatic skin lesions compared to epidermis of normal skin.

In another study done by Rashed et al[195] ,there was strong VEGF expression in epidermis(mean46.1+/-19.66) and a moderate expression in vessels and inflammatory infiltrates(mean 19+/-5.4 and 8+/-2.16).VEGF

expression was significantly higher in skin of psoriasis cases compared to normal healthy controls. In a similar study conducted by Kim YG et al[196] the expression of VEGF was significantly enhanced in skin of psoriasis cases compared normal healthy controls.

The above studies correlates with our study and also shows that VEGF promotes endothelial cell survival , promotes new blood vessel formation and thus plays a significant role in pathogenic basis of psoriasis. It appears to be one of the important factor in pathomechanism of psoriasis. Bhushan et al reported that VEGF was mainly produced by epidermal keratinocytes compared to fibroblast[130].

In the present study there was significant correlation between the expression of VEGF with PASI score.($r=0.944$, $p=<0.05$)

In a study conducted by Siaw – Cheok[9] et al there was no significant correlation between PASI score and VEGF($p=0.232$).

In the present study expression of CD 34 was observed in all samples of psoriasis with variable intensities , whereas CD 34 expression in controls was weak. There was significantly higher expression of CD 34 in cases compared to controls which is statistically significant($p=<0.01$).

In a study done by Amin et al[197] ,they observed that CD 34 expression was higher in cases than controls with a statistically significant difference(Z=2.1,p=0.04) and expression of CD 34 was significantly higher in psoriatic lesion skin compared to non lesional skin. In another study by Gupta S et al[198] showed that microvascular staining in dermis of psoriatic skin lesions was higher. Also microvessel density was higher in skin of psoriasis(71.28+/- 40.05).

A study conducted by Barton et al[199] showed that there is higher endothelial volume and volume of lumen in the skin of psoriatic lesions compared to non lesional skin of psoriasis and healthy skin of controls. In a study done by Simonetti O et al[7], CD 34 was expressed significantly higher in skin of psoriatic lesions compared to controls.(19.15+/-12.61 vs 3.0+/- 0.23; p=0.04)

The above studies supports our observation and indicates that in psoriasis there is vascular proliferation, tortuosity and elongation of vessels in response to inflammation reflected by increased microvessel density.

In the present study there was significant correlation between the expression of CD 34 with PASI score(r=0.942;p=<0.05). In a similar study by Gujiao BI et al[200] indicated that CD 34 expression was increased in vascular endothelial cells in dermis of psoriatic skin lesions

and might be related to severity of psoriasis.CD34 might be involved in adhesion and migration of inflammatory cells.

In the present study expression of vWFr both in lesional skin and control skin was weak. There was no statistically significant difference(p=>0.05). In a similar study conducted by Siaw – Cheok[7] et al there was no expression of vWFr in psoriatic cases. In the present study there was no statistically significant correlation between PASI Score and vWFr(r=0.024;p=>0.05). In a similar study conducted by Siaw – Cheok[7] et al there was no significant correlation between PASI score and vWFr (p=0.169).

The above studies indicates that angiogenesis plays a key and central role in pathogenesis of psoriasis. Other evidences which support that angiogenesis is seen in psoriasis are the following

I. enhanced blood flow in psoriasis skin biopsy using laser Doppler fluxmetry
II. increased microvasculature in the psoriatic skin lesion seen in autoradiograph and
III. the ultrastructural studies which shows tortuous and elongated capillary loops in lesional psoriatic skin.

Various other studies are being done elsewhere regarding immunohistochemical expression of angiogenesis markers and angiogenic factors in psoriatic skin lesions. It includes nerve growth factor, survivin, iNOS, basic fibroblast factor[195] and a pleiotropic osteopontin[197].

Summary and Conclusion

SUMMARY

Psoriasis is a chronic inflammatory dermatological condition which is a chronic disabling disease. Both the keratinocytes and T lymphocytes secrete these angiogenic factors which is responsible for neovascularisation in psoriasis. Angiogenesis and inflammatory infiltration might be interdependent in psoriasis. The present study was undertaken to analyse to analyse immunohistochemical expression of angiogenic factors Vascular endothelial growth factor, Von wille brand factor and CD 34 in skin biopsy of patients with psoriasis vulgaris and controls and to correlate with psoriasis area and severity clinical index(PASI SCORE).

VEGF expression in epidermis was significantly higher in cases when compared to control skin (p=<o.o1).CD 34 expression was significantly upregulated in cases when compared to controls.($p<0.01$). Whereas only weak expression of vonwillebrand factor was observed in both cases and controls. Significant correlation between the expression of VEGF and PASI score($r=0.944;p<0.05$), and expression of CD 34 and PASI score was observed($r=0.942;p<0.05$).

CONCLUSION

The present study titled as “Differential expression of angiogenic factors in skin of patients with psoriasis vulgaris” was conducted in Department of Pathology from April 2013 to July 2014”.

Our study demonstrated significantly higher expression of angiogenic factor – VEGF(vascular endothelial growth factor) and angiogenesis marker CD 34 in psoriatic skin lesions. But expression of von willebrand factor was not statistically significant. Significant correlation was also noted between PASI score and expression of VEGF and CD 34. Hence antiangiogenic therapy may be useful therapeutic approach in psoriasis.

Various other studies are being done elsewhere regarding immunohistochemical expression of angiogenesis markers and angiogenic factors in psoriatic skin lesions. It includes nerve growth factor, survivin, iNOS, basic fibroblast growth factor and a pleiotropic osteopontin

Future research with larger samples could be done to analyse in detail the angiogenic pathways for development of targeted anti-angiogenic therapy which might be beneficial for this chronic disabling disease.

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Annexure - I

ANNEXURE - I

PROFORMA

(I)PATIENT INFORMATION

- NAME
- AGE(YEARS)
- SEX
- IP/OP NUMBER
- OCCUPATION
- ADDRESS

(II)CLINICAL INFORMATION

- DURATION OF LESIONS
- SITE OF DISTRIBUTION
- AREA OF DISTRIBUTION
- FEATURES OF LESION- ERYTHEMA,INDURATION,SCALING
- PSORIATIC AREA AND SEVERITY INDEX
- TREATMENT TAKEN OR TAKEN; IF TAKEN – DURATION OF TREATMENT , SYSTEMIC OR TOPICAL

(III)SITE OF BIOPSY SPECIMEN ; NUMBER AND SIZE OF BIOPSY SPECIMEN

(IV)MICROSCOPIC FINDINGS

- H& E DIAGNOSIS
- IHC FINDINGS

CONSENT FORM

Dr..S.Lakshna, postgraduate student in the department of pathology, Coimbatore Medical College is conducting a study on **"DIFFERENTIAL EXPRESSION OF ANGIOGENIC FACTORS IN SKIN OF PATIENTS WITH PSORIASIS VULGARIS".** A skin biopsy involves removal of a small piece of skin under local anaesthesia.The piece of skin is then processed and examined under a microscope to obtain diagnostic information or is tested for other studies. I have been informed ,to my satisfaction regarding the nature of procedure. The data used herein may be used for research and publication. The data used herein may be used for research and publication.

Name :

Place :

Signature :

ஒப்புதல் படிவம்

டாக்டர். லக்ஷணா முதுகலை பட்டதாரி (நோய் குறியியல் துறை, கோயமுத்தூர் மருத்துவ கல்லூரி,) ஆய்வு **"டி.ஃ.வ்ரேன்ஸியல் எக்ஸ்பிரசன் ஆ.ஃ.ப் ஆஞ்சீயேஜீனிக் பேக்டர்ஸ் இன் ஸ்கின் வித் செரியஸிஸ் வல்கேரிஸ்"** மேற்கொண்டிருக்கிறார். இதற்காக என் தோலிலிருந்து சிறு துண்டு சதையை எடுத்து திசு பரிசோதனைக்கு அனுப்பப்படுகிறது. இந்த ஆராய்ச்சி முடிவுகள் படிப்புக்கும், ஆய்வுக்கு எடுத்துக் கொள்ளலாம். இதற்கு நான் முழுமனதாக சம்மதிக்கின்றேன்.

பெயர் :

இடம் :

கையொப்பம் :

Annexure - II

ANNEXURE - II

MASTER CHART FOR CASES

S.NO	HPE NO	IP/OP NO	AGE	SEX	PASI Score	CD 34	VEGF	VWFr.
1	385/13	461035	30	F	16.4	2+	2+	1+
2	355/13	41299	35	M	17.2	2+	3+	1+
3	375/13	40910	33	M	17.4	2+	3+	1+
4	582/13	40898	48	F	14.6	2+	2+	1+
5	583/13	3851	53	F	16.4	2+	3+	NEG
6	682/13	32554	52	M	18.8	3+	3+	1+
7	1180/13	38505	55	M	18	2+	3+	1+
8	1617/13	37205	47	M	18.8	3+	3+	NEG
9	1588/13	45944	29	F	16.4	2+	2+	NEG
10	2749/13	46407	63	F	17.2	2+	3+	NEG
11	2843/13	6684	55	F	19.6	3+	3+	NEG
12	2845/13	6189	45	M	18	3+	3+	1+
13	2221/13	39179	47	F	16.4	2+	3+	NEG
14	2222/13	38487	38	M	17.2	2+	3+	NEG
15	900/13	38891	31	F	17.4	3+	3+	NEG
16	971 /13	40582	27	F	13.8	2+	2+	1+

S.NO	HPE NO	IP/OP NO	AGE	SEX	PASI Score	CD 34	VEGF	VWFr.
17	335/14	3880	43	F	14.8	2+	3+	NEG
18	679/14	47001	42	M	14	2+	3+	NEG
19	784/14	41511	44	M	15.2	2+	3+	1+
20	1048/14	40200	56	F	14	2+	3+	NEG
21	1096/14	41904	58	M	13.8	2+	2+	NEG
22	2100/14	35611	49	M	14	2+	2+	NEG
23	2425/14	41322	44	M	16.4	2+	3+	NEG
24	1495/14	13891	51	F	14.6	2+	2+	NEG
25	1121/14	50543	50	M	19.6	3+	3+	NEG
26	1505/14	41543	39	F	18	3+	3+	1+
27	1872/14	32653	49	M	14.08	2+	3+	NEG
28	1647/14	38912	59	M	14.06	2+	2+	1+
29	1800/14	24890	38	M	13.8	2+	2+	NEG
30	2639/14	26765	48	F	13.8	2+	2+	NEG
31	3011/14	24797	36	M	17.4	3+	3+	NEG
32	3233/14	44645	47	M	17.8	3+	2+	NEG

VEGF AND vWFr - basal layer only - 1+ ; lower half of the epidermis- 2+; whole epidermis - 3+ ;

CD 34- Mild (4-10 capillaries) 1+; moderate (11-20 capillaries)2+; severe (21-28 capillaries)3+

MASTERCHART FOR CONTROLS

S.NO	HPE NO	IP/OP NO	Age	PASI Score	Sex	VEGF	CD34	VWFr.
1.	1760/13	9959	31	NA	F	neg	neg	neg
2.	1833/13	11544	36	NA	M	neg	neg	neg
3.	842/13	131652	34	NA	M	neg	neg	neg
4.	908/13	12536	47	NA	F	1+	1+	1+
5.	2006/13	5665	54	NA	F	neg	neg	neg
6.	2053/13	10209	53	NA	M	neg	neg	1+
7.	977/13	9418	56	NA	M	neg	1+	neg
8.	1076/13	6278	46	NA	M	1+	neg	1+
9.	947/13	10932	28	NA	F	neg	neg	neg
10.	1200/13	11512	61	NA	F	neg	1+	neg
11.	1406/13	9583	53	NA	F	1+	neg	1+
12.	1411/13	8079	45	NA	M	neg	neg	neg
13.	1860/13	9667	48	NA	F	neg	1+	neg
14.	3626/13	7997	38	NA	M	neg	neg	1+
15.	3711/13	5957	30	NA	F	neg	neg	neg

S.NO	HPE NO	IP/OP NO	Age	PASI Score	Sex	VEGF	CD34	VWFr.
16.	559/14	10930	26	NA	F	1+	1+	neg
17.	624/14	240221	42	NA	F	neg	neg	neg
18.	61/14	2473	43	NA	M	neg	neg	1+
19.	920/14	19224	41	NA	M	1+	neg	neg
20.	115/14	10010	54	NA	F	neg	1+	neg
21.	509/14	18231	58	NA	M	neg	neg	1+
22.	337/14	18555	56	NA	M	neg	1+	neg
23.	429/14	16838	43	NA	F	1+	neg	1+
24.	278/14	18878	47	NA	M	neg	neg	neg
25.	211/14	18902	57	NA	M	neg	neg	neg
26.	1119/14	10786	58	NA	M	neg	neg	1+
27.	1178/14	243977	59	NA	F	1+	neg	neg
28.	2760/14	18162	55	NA	M	neg	neg	neg
29.	2955/14	20766	49	NA	F	neg	neg	1+
30.	3122/14	240218	58	NA	M	neg	neg	neg

VEGF AND vWFr - basal layer only - 1+ ; lower half of the epidermis- 2+; whole epidermis - 3+ ;

CD 34- Mild (4-10 capillaries) 1+; moderate (11-20 capillaries)2+; severe (21-28 capillaries)3+

ABBREVIATIONS FOR MASTER CHART

M	–	MALE
F	–	FEMALE
NA	–	NOT APPLICABLE
VEGF	–	VASCULAR ENDOTHELIAL; GROWTH FACTOR
vWFr	–	VON WILLEBRAND FACTOR
PASI SCORE	–	PSORIASIS AREA SEVERITY INDEX

Annexure - III

ANNEXURE III

ABBREVIATIONS

VEGF	–	Vascular endothelial growth factor
Th	–	T helper cell
VWFr	–	Vonwillebrand factor
PASI SCORE	–	Psoriasis area severity index
IL	–	Interleukin
HLA	–	Human leucocyte antigen
TNF alpha	–	Tumor necrosis factor alpha
IFN gamma	–	Interferon gamma
NSAIDs	–	Nonsteroidal anti inflammatory drugs
APC	–	antigen– presenting cells
KGF	–	keratinocyte growth factor
TCR	–	T cell receptor
MHC	–	Major histo compatibility complex
GMCSF	–	Granulocyte macrophage colony stimulating factor
EGF	–	Epidermal growth factor
NFkb	–	Nuclear factor kappa beta
LFA	–	Lymphocyte function associated antigen
ICAM	–	Intercellular adhesion molecule
TSP	–	Thrombospondin

ANG	–	angiopoietin
HIF	–	Hypoxia inducible factor
EC	–	endothelial cell
ECM	–	extracellular matrix
MMP	–	Matrixmetalloproteinases
TGF alpha	–	Transforming growth factor
VEGFR	–	Vascular endothelial growth factor
RNA	–	Ribonucleic acid
HIV	–	Human immunodeficiency virus
AIDS	–	Acquired immunodeficiency virus
LCH	–	Langerhans cell histiocytosis
I	–	Induration
E	–	Erythema
S	–	Scaling
H & E	–	Haematoxylin and eosin
DAB	–	3,3'– diaminobenzidine
PBS	–	Phosphate buffer saline